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In memoriam

CONSIDERATIONS ON SOME ELEMENTARY FEATURES OF MITOSIS

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I. INTRODUCTION.

A new discussion of the elementary and generally well-known features of the chromosome movements at cell division might scarcely seem capable of making any essential contribution to the understanding of the mechanism for these movements. These features have already been discussed so often and so many different hypotheses have been advanced to explain them (*cf.* SCHRADER, 1944) that the subject might seem exhausted by now.

But renewed attacks on the problems of the chromosome movements by the present writer and co-workers, involving *inter alia* a study of trivalents and T-chromosomes, have brought out new interpretations of some aspects of the chromosome movements (ÖSTERGREN, 1945 a, b, 1947, 1949 a, b, c; ÖSTERGREN and PRAKKEN, 1946; BÖÖK, 1945; PRAKKEN, 1948). In these papers there was presented a hypothesis concerning the forces producing the movements of the chromosomes to the equator during prometaphase and to the poles during anaphase. This hypothesis was, however, described in a somewhat condensed form, and for this reason it seems justified to supplement these papers with a more detailed description of the hypothesis.

It is suitable to present this description of the hypothesis in connection with a discussion of the *elementary features* of the chromosome movements. In a few cases in which it is considered useful some important *details* of the chromosome behaviour will also be discussed, but otherwise the treatment of the details of the process will be reserved for other papers.

II. METHOD OF ANALYSIS.

The influences acting on the chromosomes during mitosis are of many different kinds. In the case of some of them it is an easy matter to find out the nature of the influence in question. Their analysis does

not offer any special problem. Thus, when chromosome regions which are lying outside the spindle appear to be moved by cytoplasmic currents (as observed by BĚLAŘ, 1929), we need not doubt that this is actually the case.

In the case of many other influences acting on the chromosomes it is much more difficult to find out their real nature. We can be safe when we conclude that the *deformation* of the chromosomes during mitosis is due to *forces* acting on them (although in some special case there might be a difficulty in judging whether a change in shape is due to an external influence or to internal chromosome changes, such as an increased spiralization). Even if, from evidence of this kind and for other reasons, we can safely conclude the *existence* of many forces working during mitosis, it is often fairly difficult to discover the *nature* of the forces in question. The best thing we can do in these cases is undoubtedly to put the speculations on the nature of the forces in the background, and to concentrate instead on the extraction of as much knowledge as possible concerning the *properties* of the forces directly from the events of mitosis. It is possible to demonstrate conclusively the existence and the characteristic properties of a number of influences acting on the chromosomes at mitosis, although the physicochemical nature of these influences may be quite unknown. The result of this method of analysis will be a number of *empirical regularities* of chromosome behaviour, *i. e.* regularities concerning which we conclude from our data (with a higher or lower degree of safety) that they *exist*, without having any knowledge concerning their *nature*.

The forces which cause the most important events during mitosis, namely the movement to the metaphase plate during prometaphase and that to the poles during anaphase, belong to the group of effects that must be analysed in this way. It does not seem advisable to introduce in the analysis of the properties of these forces some more or less preconceived ideas concerning the nature of the forces, such as the assumption that they must be of an electrical nature, and to let this introduction be followed by a re-description of the events of mitosis in terms of such a preconceived idea, a method which seems to have been applied by some authors.

This does not mean that we should refrain from making speculations on the mechanical nature of the forces acting during mitosis, only that we should be cautious not to let these uncertain speculations exert an undue influence on the interpretation of the properties of the forces.

The present discussion of the elementary features of mitosis will be conducted in terms of an extraction of empirical factors of chromosome behaviour, and speculations on the mechanical nature of the forces will be omitted from the present paper. An effort to give an interpretation of the physical nature of the forces acting on the chromosomes has been presented elsewhere (ÖSTERGREN, 1949 b, pp. 460—466).

The discussion submitted below will consist of a consideration of various *alternatives* that may be suggested concerning the *properties* of the forces acting on the chromosomes. Some of these alternatives have already been suggested by others, who then also presented hypotheses concerning the mechanical nature of the forces assumed in these alternatives (pp. 9—12 and 16, below). It might seem to be an important argument in favour of these alternatives that the forces assumed by them appear to be explicable in this simple way by well-known physical effects. For this reason the present discussion has also been extended to include a few physical aspects of these hypotheses. But, as will appear below, the physical side of these hypotheses cannot be maintained. Thus, the alternatives represented by these hypotheses have no advantage in this respect over the other alternatives.

The biological system of the spindle and the chromosomes is obviously a very complicated one. It can by no means be taken for granted that the mechanical factors working in this system are of a simple and easily analysable kind. The correctness of the empirical regularities of behaviour that can be extracted from the events of mitosis cannot be tested by investigating whether they are in agreement or not with some simple and well-known physical effects. (Naturally with the exception that our regularities must not disagree with certain fundamental principles. That need scarcely be mentioned.)

III. ATTRACTIONS OR REPULSIONS.

In the following discussion the terms »attraction» and »repulsion» will often be used. »Attraction to the pole» is used simply to describe the situation in which a force is striving to move a body to the pole, and »repulsion from the pole» the situation in which a force is endeavouring to move a body away from this spindle region. These terms do not involve any assumption that there must be an active engagement of a polar body in the process (note that I am writing »attraction *to* the pole» and not »attraction *by* the pole»). In some individual cases it may be possible that some special analysis will demonstrate an active

engagement of a polar body (centrosome), in other cases (many higher plants) there is even lacking a morphologically distinct pole (barrel-shaped spindles). Such differences can be disregarded in the present connection, as the general course of mitosis is the same. Instead of writing »attraction to the pole» we could write »attraction to the polar region» in the case of barrel-shaped spindles.

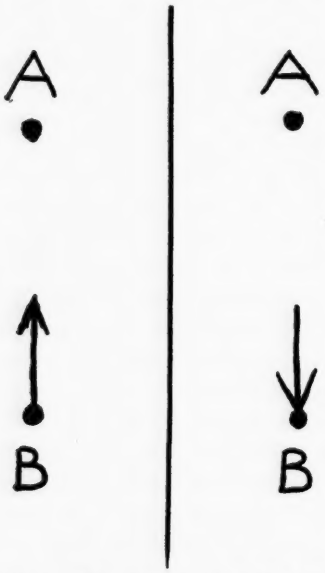


Fig. 1. The method used in the present paper of describing attractions and repulsions by means of arrows. In the left figure *B* is attracted to *A*, in the right figure *B* is repelled from *A*.

Fig. 1 demonstrates the method adopted in the present paper of giving a schematical representation of attractions and repulsions by means of arrows. (The same method will also be used in the other papers of the present series of works, except where some special features of the figure make it unsuitable, as in Fig. 23 of ÖSTERGREN, 1949 b. In the case of exceptions the situation will be noted by special explanation.)

Even if the forces acting on the kinetochores [1] may result through an interaction of these chromosome regions with the spindle as a whole, it is suitable to select the spindle poles as points of reference in the description of the spindle—kinetochore interaction. The fact that the anaphase movements are directed to and are finished at the poles demonstrates that these regions have a special significance in the force system of the spindle.

Probably the forces influencing the kinetochores relative to the poles do not act along straight lines, but follow more or less the curved fibre arrangement of the spindle. This situation need not be an obstacle to a descriptive treatment of the forces as attractions and repulsions relative to the poles.

There are only six conceivable types of attractions and repulsions

[1] In his earlier papers the present writer has used the term »centromere», but a consideration of this terminological question has now led him to the opinion that »kinetochore» should be a better term. In this question I agree with LIMA-DE-FARIA (1949, p. 422).

that could act on a kinetochore relative to a spindle pole, *viz.*: (a) a force of *attraction decreasing* with increasing distance, (b) a force of *repulsion decreasing* with increasing distance, (c) a force of *attraction increasing* with increasing distance, (d) a force of *repulsion increasing* with increasing distance, (e) a force of *attraction independent* of distance (*i. e.* constant), and finally (f) a force of *repulsion independent* of distance.

It should be noticed that these expressions »attraction increasing with increasing distance», *etc.* are intended to describe only the *direction* of the change in the force with the distance, and that they consequently must not be taken to describe any quantitative relations between the force and the distance, such as direct proportionality. The hypothesis of ÖSTERGREN (1945 a), which involved an expression of this kind, has been misunderstood in this respect by HUGHES and SWANN (1948, p. 66).

It may be useful to give all these six alternatives [(a)—(f) above] an equal chance when we *start* the present discussion of the forces acting on the chromosomes during mitosis. It cannot be justifiable to state that, as the forces most probably are electrostatic, we need only consider alternatives (a) and (b), or to make some similar restriction founded on a preconceived idea concerning the nature of the forces.

As the spindle has two poles, there are two further alternatives to be regarded concerning the interrelation of kinetochores and spindle poles, *viz.* either that a kinetochore behaves in the same way towards both poles, or that it behaves in a different manner towards them. There is evidence that an anaphase kinetochore has different properties on different sides: it has what may be called a front side and a back side. Thus, an anaphase kinetochore has a so-called chromosomal spindle fibre attached to that side of it which it turns towards the pole to which it is moving (*cf.*, *e. g.*, SCHRADER, 1939, 1944). Under these circumstances it would even seem more probable that the forces of the spindle influence the anaphase kinetochore in an asymmetric way than that the influence is of the same nature in both the two poleward directions. It would not be surprising to find, *e. g.*, that such a kinetochore may be attracted only towards that pole towards which its front side is turned.

The elementary features of the chromosome movements at mitosis and meiosis are that they move to the equator during prometaphase, where they remain during metaphase, after which the daughter (or partner) chromosomes move to the poles during anaphase. In this course of events the kinetochores are the active components and the chromosome arms are passively dragged along by the kinetochores. Furthermore, in the bivalents at the first metaphase of meiosis the two partner

kinetochores arrange themselves on a line parallel to the spindle axis (the so-called co-orientation).

The prometaphase movements, which lead to the metaphase arrangement of the chromosomes in an equatorial plate, must obviously be due to forces influencing the kinetochores relative to the spindle, so forces of that kind must in any case be assumed. In the anaphase movement there could conceivably also be suggested forces of repulsion between the separating kinetochores. If, however, the forces that we *must* assume to explain the prometaphase movements are of a kind capable of also producing the anaphase movements, we shall only introduce an unnecessary complication by making the additional assumption of a repulsion between the anaphase kinetochores, unless there is special evidence that such forces are really *also* working.

We can be fairly safe in concluding that the metaphase stage is characterized by an *equilibrated position* of the chromosomes on the equator. The concept of the metaphase equilibrium implies that the chromosomes remain on the equator because they are subjected to opposing influences that balance one another. As an alternative interpretation it might be suggested that the chromosomes remain on the equator simply because they are not subjected to any forces at all; a mechanism has brought them there and stopped working when they have arrived at the equator. The prometaphase events shed some light on the probability of these two alternatives. It is a characteristic feature of the prometaphase that the movements to the plate are asynchronous, some chromosomes at mitosis (or bivalents at the first division of meiosis) have reached the equator before the others and remain there while the others still perform the prometaphase movements (for mitosis, see SCHAEDE, 1938, Figs. 1—2; for meiosis, see HUGHES-SCHRADER, 1943, 1947). In such cells (Fig. 2) it is clear that the chromosomes on one side of the equator (as, *e. g.*, the bivalent *C* in Fig. 2) are influenced by forces moving them towards the equator just as is the case with the chromosomes on the opposite side of the equator (*e. g.*, *B* in Fig. 2). Then it appears as most probable that those chromosomes that have reached the equatorial position in such a cell (*A* in Fig. 2) are influenced by the same forces, which then balance one another. It is of interest in connection with this idea of opposing forces working on the chromosomes in the equatorial plate that HUGHES and SWANN found, in their film of mitosis in living chick cells, that the chromosomes oscillated in the longitudinal direction of the spindle during metaphase (1948, pp. 52 and 59). These authors state: »It almost seems as if an

equilibrium between opposing forces is maintained, until the balance is finally lost at anaphase». Similar observations were reported by LEWIS (1939), who also studied mitosis in tissue culture cells, and BĚLAŘ (1929) studying meiosis in living spermatocytes of a grasshopper reports a slow movement of the bivalents to and fro across the metaphase plate in the longitudinal direction of the spindle (*l. c.*, p. 376 and Figs. 40—43 on Plate III). Furthermore, it should be mentioned that the phenomena of the so-called transverse equilibria (ÖSTERGREN, 1945 b, and unpublished) suggest the same idea of a balance of forces working on the chromosomes towards the two opposite spindle poles.

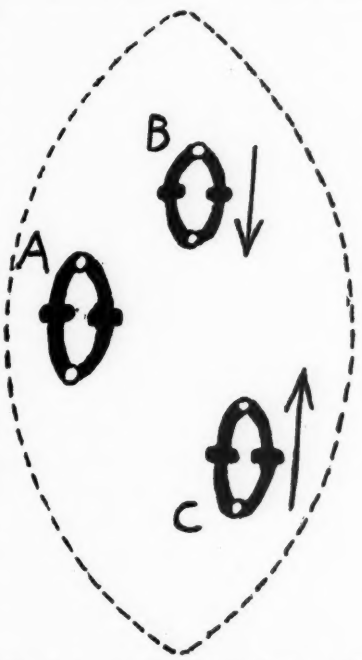
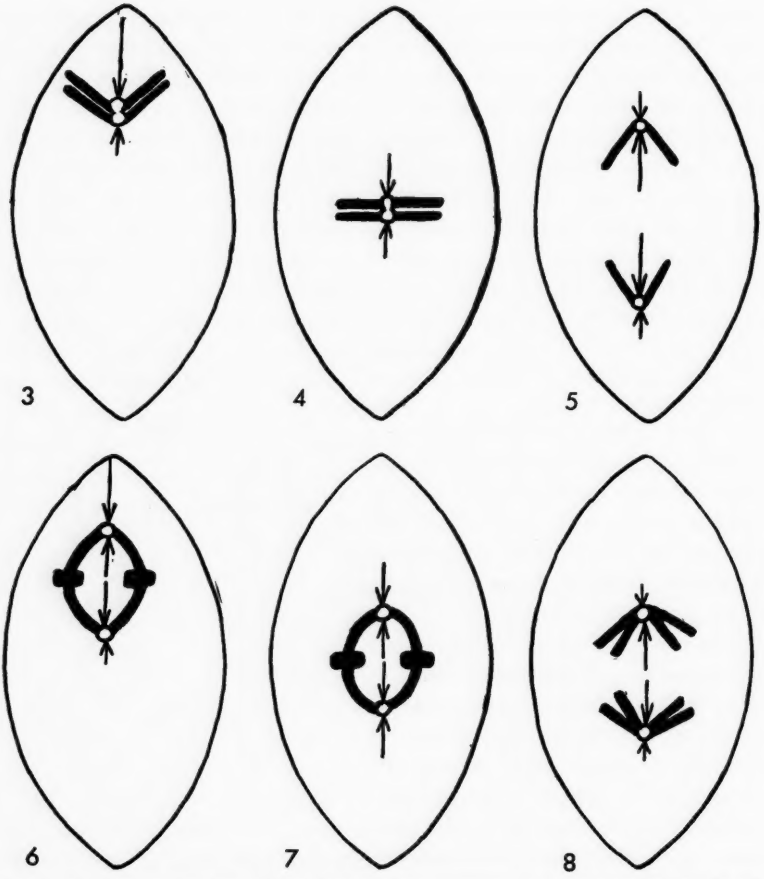


Fig. 2. Some characteristic features of prometaphase schematical'y demonstrated on a cell from the first division of meiosis. The arrows indicate the direction of the movement.

The idea of metaphase as an equilibrium between opposing influences is not a new one. It is of fairly common occurrence in the literature on mitosis. A well-known hypothesis in which this is assumed is that by DARLINGTON (*e. g.* 1937), and similar ideas have also been suggested by many authors before him. Many of the old adherents of the traction fibre hypothesis had the opinion that the chromosomes were pulled at by traction fibres in both the two poleward directions during metaphase.

What alternatives of those suggested above (p. 5) would be able to explain the stabilization of the chromosomes in an equatorial equilibrium half-way between the two poles? First it can be concluded that the metaphase kinetochore of mitosis must be influenced in the same manner relative to both poles. If the influence had been different with regard to the two poles, the equilibrium would, at least, not have been half-way between the two poles.

Alternatives (e) and (f) of those enumerated above (p. 5) imply that the forces do not change with the position of the chromo-



Figs. 3—8. The mechanism of the chromosome movements as assumed in the hypothesis of DARLINGTON (*e. g.* 1937). — Figs. 3—5. Ordinary mitosis. — Figs. 6—8. The first division of meiosis.

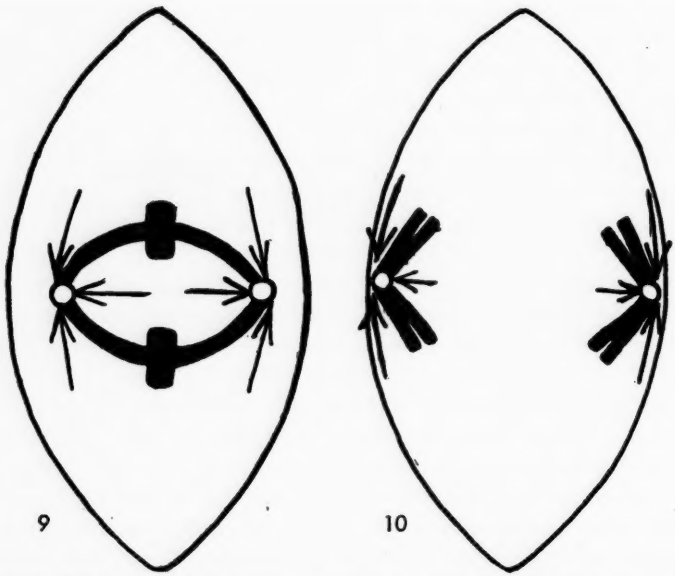
somes. They cannot make any position (such as the equator) preferred by the kinetochores and can be dismissed from further discussion. It is true that alternatives (a) and (d) imply that the force acting on a metaphase kinetochore of mitosis is of the same magnitude in the direction of the two opposite poles, if the kinetochore is lying exactly half-way between the two poles. But this would not be a stable position in the case of these two alternatives. The slightest shift towards the one

side or the other would start a movement away from the equator, and naturally forces of this kind would never bring about a movement to the equator, as in prometaphase. Thus, these alternatives do not deserve further consideration.

The two alternatives (b) and (c), on the other hand, give an easy explanation of an equilibrium half-way between the two poles. Alternative (b) is that adopted by DARLINGTON (1936, 1937, 1939) in his electrostatic theory. This hypothesis is described schematically in Figs. 3—5, in the way in which it is supposed to work by its originator. The kinetochores move to the equator because they are repelled by the poles, and the two daughter kinetochores separate during anaphase because they repel one another. The repulsion of the kinetochores from the poles is supposed to decrease during anaphase, and the separation of the chromosomes is facilitated by the stretching of the middle piece of the spindle (the »Stemmkörper» of BĚLAŘ, *e. g.* 1929). The two kinetochores of a bivalent at meiosis are supposed to repel one another in the same way as the daughter kinetochores of a somatic chromosome do during anaphase (Figs. 6—8). The repulsions are supposed to be due to strong electric charges localized at the kinetochores and the spindle poles. The charges at the poles are supposed to decrease during anaphase, and for this reason the chromosomes would be able to approach the poles.

An important argument against this hypothesis of DARLINGTON lies in the fact that a bivalent at meiosis is co-oriented lengthwise on the spindle at metaphase, with its two kinetochores as close as possible to the poles. Another closely related argument is found in the fact that the movement of the anaphase chromosomes is definitely directed towards the poles, and not only away from one another. DARLINGTON's hypothesis is also criticized by CORNMAN (1944) because of this character of the anaphase movement. On the basis of the assumption that the poles repel the kinetochores we should not expect the bivalents to orient in the manner found at first metaphase, but instead we would expect them to orient crosswise on the spindle, and we would also expect the separating anaphase chromosomes to move in a direction perpendicular to the spindle axis (Figs. 9—10).

Evidence against this hypothesis of a kind that seems even more important is given by the behaviour of the chromosomes in cells having a mixture of paired and unpaired chromosomes at the first division of meiosis. In such cases it can be found (*e. g.* personal observations on various materials of the Triticinæ) that some univalents move to the



Figs. 9—10. The events that one expects to take place on the basis of the repulsions assumed in DARLINGTON's hypothesis. — Fig. 9. At first metaphase the bivalents will lie down in the equatorial plane. — Fig. 10. The direction of the anaphase movement.

poles already during prometaphase, when the bivalents move to the equator. Such univalents can occupy positions with their kinetochores closely approached to the poles. This behaviour demonstrates that the movement of the bivalents to the equator cannot be due to a repulsion of electrically charged kinetochores from spindle poles that have a similar charge. If it had been so, the univalents should also have been repelled.

The above evidence against the hypothesis of DARLINGTON (1936, 1937, 1939) seems conclusive. It should scarcely be necessary to discuss it further. But some of the difficulties of this hypothesis (*viz.* the direction of the co-orientation of the bivalents and that of the anaphase movement) were realized by DARLINGTON himself, and it may be of interest to consider the accessory assumptions by which he strives to overcome these difficulties.

DARLINGTON points out that the spindle is anisotropic (an opinion that undoubtedly is correct), and he suggests that because of this property it will transfer electrical repulsions more strongly in one direction than in the others, a suggestion that is quite reasonable. The electrical repulsions would be transferred more strongly lengthwise than crosswise on the spindle, he supposes. In such an anisotropic field it is obvious that a bivalent, with two strongly repelling kinetochores, will have a different potential energy depending on whether it is oriented crosswise or length-

wise in the field, and it is also clear that it will orient itself spontaneously in that position which gives it a minimum of potential energy. These relations were realized by DARLINGTON, and he suggested that the bivalent assumed that orientation which made the force of repulsion between its two kinetochores as strong as possible. This orientation would, he assumed, correspond to a minimum of potential energy. Undoubtedly, the situation found during nuclear division has a certain similarity to what would be expected on the basis of these assumptions. The two partner kinetochores of each bivalent at first metaphase are subjected to quite strong forces striving to separate them, but there are no or only weak forces acting in the transverse direction of the spindle between the kinetochores belonging to different chromosome pairs.

There is, however, an important objection that must be raised against this interpretation, *viz.* that we *should not expect* the arrangement giving the *strongest* repulsion between the kinetochores to correspond to the minimum potential energy, but this would instead be expected to be the case with the arrangement giving the *weakest* repulsion. If it had been a matter of attraction, the bivalent would have oriented in such a manner that the force was transferred as efficiently as possible, but in the case of repulsion it orients in the opposite way. This relation will be clear if we consider an »ideal» bivalent having its two kinetochores separated by a constant distance *D*, and which is placed in an anisotropic field in the two alternative orientations where the repulsion is maximal and where it is minimal. The difference in potential energy between these two arrangements can be estimated in the following way: Assume that the charged bodies (the kinetochores) are moved in an anisotropical field of infinite size, from an infinite distance apart until they reach the distance *D* from one another. This movement is for comparison assumed to be performed in both the directions in question. It is clear that it will be necessary to use more work to perform this movement when the bodies are moved in the direction where the repulsion is stronger than in the other one. Consequently, the potential energy is higher in the orientation, where the kinetochores of the bivalent exert a stronger mutual repulsion, and we should expect the bivalent to orient itself spontaneously in such a direction that the repulsion is as weak as possible.

(Perhaps it will be suggested that the potential energy of the two orientations of the bivalent might, instead, be analysed by starting with the two kinetochores in direct contact with one another, after which they are separated from this position until they reach the distance *D* from one another. This separation is accompanied by a decrease in potential energy, and the decrease is larger when the separation is performed in the direction in which the repulsion is stronger. Thus, it would seem from this reasoning that the orientation with the stronger repulsion would correspond to the minimum potential energy. But this conclusion is erroneous, because in this case the kinetochores must have had a different potential energy in the two different initial arrangements from which their separation was started.)

Thus, there is no sound physical background for the electrostatic hypothesis of DARLINGTON, and, moreover, there is evidence against it of a simple common-sense nature, *viz.* that derived from the behaviour of the univalents referred to above, which in itself would be quite sufficient to force us to give up this hypothesis.

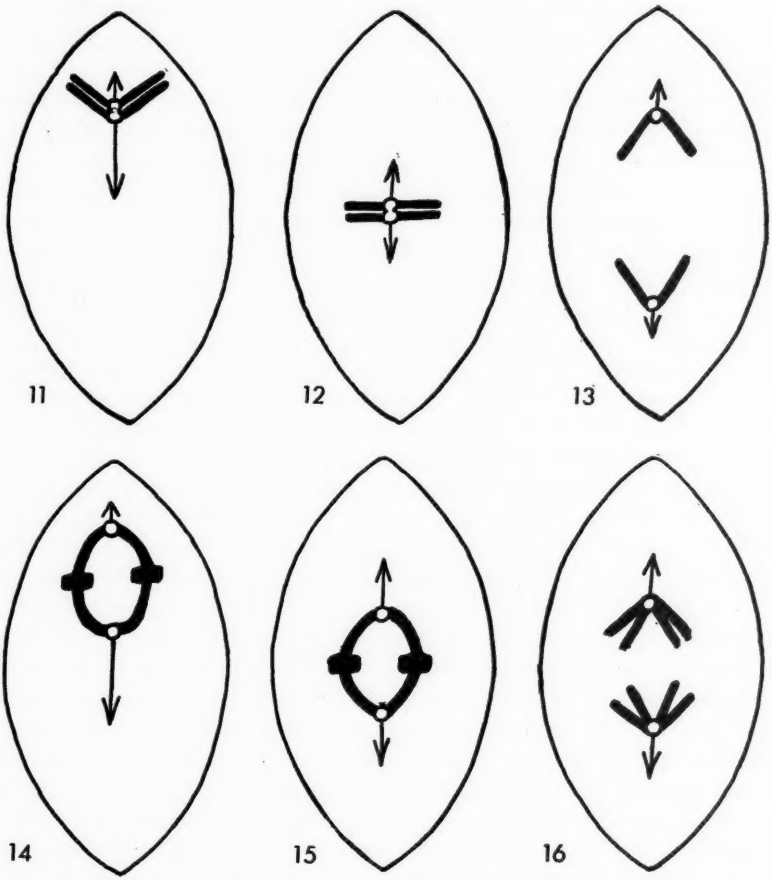
Judging from a recent paper by DARLINGTON (1947) it seems that he has to a large extent abandoned this hypothesis of his and accepted the main features of the ideas of BERNAL (1940). One may then question why so much attention has been

paid to his old speculations here. The reason is that this hypothesis has been accepted by many cytogeneticists, who probably continue to interpret their data along these lines.

It is now possible to return to the subject that was under discussion when this theoretical excursion was made. We were trying to find out what kind of *properties* the forces acting on the kinetochores must have in order to make possible the metaphase equilibrium. Alternative (b) cannot give an explanation of the metaphase arrangement of the bivalents, when applied in connection with the idea of a symmetrical interaction between spindle and kinetochores. The result of this alternative would be that the bivalents would lie down on the equatorial plate instead of co-orienting lengthwise, as they actually do. The specialized assumptions of DARLINGTON, which might have changed this situation, are impossible to maintain.

But if we instead assume, in connection with alternative (b), that each anaphase kinetochore at mitosis or partner kinetochore at the first division of meiosis is repelled in this manner from *one pole* only, then the situation will be a different one. Before discussing this idea, however, it may be useful to discuss alternative (c), *viz.* that the kinetochores are attracted to the poles by forces increasing with the distance.

It is obvious that such forces as are assumed in alternative (c) easily explain the movement to the equator of a mitotic chromosome and also its equilibrium position on the equator. If each kinetochore were influenced by forces of this kind towards both poles, however, the hypothesis would be incompatible with the co-orientation found in the bivalents. In this case we should also expect the bivalents to lie down on the spindle equator. But if we assume instead that these *forces of attraction are active between the anaphase kinetochore* (equal to half kinetochore at metaphase of mitosis) and *only one of the poles, viz.* that pole towards which the »front side» of the kinetochore is turned, then we can easily explain the characteristic features of the chromosome movements. The events of mitosis such as they are interpreted on the basis of this hypothesis are described by Figs. 11—13. This is the hypothesis suggested by ÖSTERGREN (1945 a, b; 1949 a, b, c; *cf.* also ÖSTERGREN and PRAKKEN, 1946; PRAKKEN, 1948). The metaphase kinetochore at mitosis behaves simply as the sum of two anaphase kinetochores attached to one another with their akinetic sides. The two halves of such a metaphase kinetochore are oriented with their kinetic sides (or »front sides») towards opposite spindle poles. As the metaphase kinetochore is attracted by both poles and more strongly by the more distant one,



Figs. 11—16. The mechanism of the chromosome movements as assumed in the hypothesis of the present writer (ÖSTERGREN, 1945 a, b). — Figs. 11—13. Ordinary mitosis. — Figs. 14—16. The first division of meiosis. — *Note*: The manner used here of drawing the kinetochore is not intended to explain in any way its structure or size, but only to indicate the presence of a specialized body at this position in the chromosome.

it will move to the equator, where the two attractions balance and the position is equilibrated. After the division of the metaphase kinetochore, each of the two anaphase kinetochores moves to that pole to which it is attracted (the pole towards which it is turned). This hypothesis has

the advantage that it makes it possible to explain both the movement *to* the equator during prometaphase and the movement *away* from the equator during anaphase by *the same mechanism.*

The partner kinetochores of a bivalent at the first division of meiosis have an asymmetric structure, each one of them having a front side and a back side, *i. e.* their structure is closely related to that of anaphase kinetochores at mitosis. They have their front sides turned towards opposite spindle poles, and the attraction of them towards opposite poles orients the bivalent lengthwise in the spindle (the co-oriented arrangement) and brings the bivalent to the equator. When the chromatids separate at the end of metaphase because of the lapse of »chromatid attraction» (whether this is a real *attraction* or not need not be considered in this connection), then the two partner chromosomes become free from one another and move to the poles because of the continued action of the same forces of attraction that earlier brought the bivalent to the equator (Figs. 14—16). The behaviour of the chromosomes during the first division of meiosis is fairly complicated, especially the phenomena of co-orientation. These problems are treated in a separate paper by the present writer, where more details can be had by the reader (ÖSTERGREN, manuscript in preparation).

It might be asked whether there exist at all such forces in nature, *i. e.* attractions of the type that they increase in strength with an increased distance. Such forces, however, do exist. An example is the case of two bodies connected by a rubber band. The more widely the bodies are separated, the more strongly are they pulled at by the elastic band. Thus, the argument by DARLINGTON (1937, p. 533) that the forces should »on all analogy» increase in effect as the chromosomes approach the poles, cannot be maintained. The chromosomes on the spindle behave in many respects as if they were fixed to the poles by attenuated elastic bands. The old traction fibre hypothesis consequently appears to be a relatively good explanation for the chromosome movements. There is, however, also evidence that this hypothesis may be far from satisfactory, and that we may have to look for some other explanation (*e. g.* ÖSTERGREN, 1949 b). In any event, it is clear that it is unreasonable to raise the objection that it would be against some fundamental principle of nature to suggest that an attraction under certain circumstances may increase in intensity with an increased distance.

It was mentioned above that alternative (b), *i. e.* forces repelling the kinetochores from the poles decreasing in strength with an increasing distance, would give a different effect if each anaphase kinetochore

were affected in this manner only relative to one pole instead of both. As a matter of fact, this mechanism would also be able to bring about a normal orientation of the bivalents as well as a normal course of the early anaphase movement. In such a case the chromosomes would not be moved by attraction to the pole they move towards, but by repulsion from the pole they are moving away from, their reaction towards the closer pole being absent or negligible. It would seem difficult for this hypothesis to explain the fact that the movements of the chromosomes are actually *directed towards* a pole, and not merely away from the opposite one, as often can be seen in late anaphase. This idea is furthermore incompatible with the data indicating the transverse equilibria (*cf.* ÖSTERGREN, 1945 b, and unpublished).

Ultimately it is a *matter of definition* whether we speak of forces striving to move a body towards a definite point as forces of attraction that are attracting the body towards the point, or whether we speak of the body being repelled away from all other places towards this point. It should be realized that changes of such definitions do not mean actual changes of hypotheses, but only a translation of them into different language. In ordinary language, I believe, it should be stated that the kinetochore is attracted to the spindle pole.

IV. CONCLUSIONS AND ADDITIONAL REMARKS.

The anaphase movement to the poles and probably also the prometaphase movement to the equator may have many different types of force suggested for their explanation. But the characteristics of the metaphase stage, on the other hand, are compatible with only quite few types of forces. These characteristics are the equilibrium of the chromosomes in the equatorial plate and the orientation of the bivalents, which takes place in such a manner that their kinetochores are as close to the poles as possible. These data are best explained by the idea of an attraction of the kinetochores towards the spindle poles growing stronger with an increasing distance, an attraction working only towards that pole towards which the kinetochore turns its front. As the same kind of force also quite easily explains the prometaphase and the anaphase movements, it certainly appears to be the most simple course to assume that forces of this kind work all through the spindle stage of mitosis. Under these circumstances it seems that we introduce an unnecessary complication if we assume fundamental changes in the main forces between the different stages. This opinion does not exclude, of course, that there

may be some changes in the intensity of this same attraction between different stages, or that it may be supplemented in some cases by accessory factors working only during special stages, *e. g.* the anaphase elongation of the spindle.

This explanation is in good agreement with the various details of the mitotic events, as will be clear from papers that are being worked out for publication. A study of these details also reveals, as naturally was expected, that the poleward attraction mentioned above is not the only factor influencing the behaviour of the chromosomes on the spindle, although it may be considered as the most important one. This attraction was called the a-factor by ÖSTERGREN (1949 c); besides this factor the paper cited enumerates 15 other factors that influence the behaviour of the chromosomes on the spindle.

Historical notes. — Finally it should be mentioned that the present idea (ÖSTERGREN, 1945 a and b, and above) of an attraction increasing in strength with an increasing distance between kinetochores and spindle poles as the factor producing both the arrangement of the chromosomes in the metaphase plate and their anaphase movement to the poles is not entirely new. Similar considerations have been advanced earlier by some adherents to the traction fibre hypothesis (*e. g.* DRÜNER, 1895, and especially RASHEVSKY, 1941), who then assumed that the mechanism of these attraction forces were contractile fibrils. The following deserves to be cited from RASHEVSKY (1941, p. 3): »Quite regardless of the nature and mechanism of the forces present, the chromosomes will be located stably in the equatorial plane only under one of two conditions: either they are attracted to the poles by forces *which increase with distance*, or else they are repelled by the poles by forces *which decrease with distance.*» But RASHEVSKY's idea that the forces in question may be simple elastic forces does not appear very probable. The material from which he deduces a quantitative agreement of the chromosome movements with movements of the type obtained by an elastic pull is too small (*cf.* also the criticism by SCHRADER, 1944, pp. 58—60). Anaphase curves in some other studies do not lend themselves very well to this interpretation, *e. g.* those of RIS (1943) from the aphid *Tamalia.* It may seem an attractive idea to refer the chromosome movements to an already well-known physical effect such as the elasticity of fibres. But that interpretation does not appear probable when attention is paid to the recent results of ÖSTERGREN (1949 b), which demonstrate that the chromosomes move fairly easily in the transverse direction of the spindle, straight across the presumed traction fibres.

Some of the other adherents of the traction fibre idea have not discussed the metaphase and prometaphase events, but they have restricted their considerations to the anaphase movement alone. To strive at a separate analysis of the anaphase events implies that one refrains from using a lot of important information bearing on the anaphase movement, which is obtainable from the other stages of mitosis. Such a procedure will obviously strongly reduce the probability of arriving at a correct solution of the problem.

SCHRADER, discussing various alternatives in his book on mitosis (1944), does not commit himself very definitely to any special hypothesis, although one gets the impression that he considers it a serious possibility that an active extension of the spindle fibres might push the chromosomes into the metaphase arrangement (*l. c.*, pp. 43 and 49), from which they are later pulled to the poles by fibre contraction. The paper by HUGHES-SCHRADER (1947) demonstrates how far the hypothesis of the present paper has been from the minds of cytologists in general. Thus, when she finds that her material bears evidence of a poleward attraction of the kinetochores during prometaphase, she concludes that the movement of the bivalents to the equator takes place *in spite of* this poleward attraction, although it might have been natural to conclude that it takes place *because of* this attraction. Concerning the chromosomal fibres she says (*l. c*, p. 6): »The subsequent congression thus takes place against any pull exerted by them.» And on p. 18: »Whether the resumption of chromosome contraction and the movements of congression imply a waning or an over-riding of the attraction is unknown.»

In his paper of 1947 SCHRADER takes up ideas of the kind suggested in the present paper for serious consideration, at the same time still regarding it as quite probable that the spindle fibres may sometimes exert a pushing action. And it must be admitted that SCHRADER's *Brachystethus* case gives strong evidence in support of the opinion that in some special case the spindle can exert a pushing influence on the chromosomes.

SUMMARY.

A consideration of certain elementary features of the chromosome movements at mitosis, *viz.* the prometaphase movement to the equator, the metaphase equilibrium at the equator and the anaphase movement to the poles, demonstrates that these are in good agreement with the hypothesis that the kinetochores are attracted to the spindle poles by forces increasing in strength with an increasing distance, and that this attraction only acts towards that pole towards which the kinetochore turns its front side (or as it may also be called, its kinetic side). The kinetochore obviously has a special differentiation, of such a kind that the anaphase kinetochore of mitosis may be said to have a kinetic side and an akinetic side, and the mitotic metaphase kinetochore consists essentially of two anaphase kinetochores attached to one another by their akinetic sides.

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ACCESSORY CHROMOSOMES IN ANIMALS, ESPECIALLY IN POLYCELIS TENUIS

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I. INTRODUCTION WITH REMARKS ON NOMENCLATURE.

TO denote such extra chromosomes as are not homologous with those of the ordinary complement and whose number is in equilibrium in a population, several terms have been used and are still in use in the karyology of plants, where their occurrence and behaviour have been summarized by HÅKANSSON (1945), MÜNTZING (1946, 1949) and ÖSTERGREN (1947).

The earliest observation of chromosomes of this kind was made in 1906 by ANNE M. LUTZ on the coleopter *Diabrotica 12-punctata* (STEVENS, 1908), but no special name was given to them at that time.

In WHITE's summary (1945) on chromosomes of this kind — the only one dealing with animal material — the term *supernumerary* is used, which is also the oldest. It was launched by WILSON (1907) as a denomination of the chromosomes in *Hemiptera* of the genus *Metapodius* that vary in number in the population and are more or less inert and behave particularly like the *Y*-chromosome of the species. This term, however, is not very convenient, partly because the general meaning of the word applies to all forms of chromosomes that occur above those of the ordinary complement, and partly because it is not so well defined, owing to the fact that in the case of *Metapodius* — to judge by research so far — no difference can be established between *Y*-chromosomes and chromosomes of the above-mentioned kind. For the sake of greater uniformity it is to be desired that we should agree on a common denomination. HÅKANSSON (1945) takes up MCCLUNG's (1900) term *accessory chromosome* and suggests a change of its old meaning (which is what we now call *X*-chromosome in an *XO*-organism). Since the term cannot now cause any confusion and it is neutral enough to persist, it deserves to be in general use, and so I am going to use it below.

The following account of the occurrence of accessory chromosomes in animals does not claim to be quite complete or to have classified the accessory chromosomes correctly in each case. The fact is that our

knowledge of the chromosomes is often too limited for definite conclusions to be drawn about their real nature. So far, there is in animals no case of accessory chromosomes that is even approximately so well known as, for instance, the cases in rye or maize. It is to be supposed, however, that in future the real nature of accessory chromosomes will be explained from many points of view in the case of animal material as well, since undoubtedly chromosome conditions are more heterogeneous in animals than in plants.

II. LIST OF SPECIES THAT HAVE OR MAY BE SUPPOSED TO HAVE ACCESSORY CHROMOSOMES.

Asterisks denote that — *judging from the available data* — accessory chromosomes are to be found in the species. The remaining cases might have them, but another interpretation is possible or else sufficient data are missing. The number in front of each species refers to the list of literature appended.

		Plathelmintes (Turbellaria).		
1.	**Polycelis tenuis* IJIMA	*Tricladida*	*Paludicola*	
2.	**Rhynchodemus terrestris* MÜLL.	»	*Terricola*	
		Insecta.		
3.	*Ceutophilus sp.*	*Orthoptera*	*Rhaphidophoridae*	
4.	**Gryllotalpa africana* BEAUV.	»	*Gryllotalpidae.*	
5.	**Gryllotalpa gryllotalpa* L.	»	»	
6.	*Tettigidea parvipennis* HARRIS	»	*Tetrigidae*	
7.	*Hesperotettix viridis* THOMAS	»	*Acrididae*	
8.	**Camnula pellucida* SCUDD.	»	»	
9.	*Circotettix lobatus* SAUSS.	»	»	
10.	*Circotettix undulatus* THOMAS	»	»	
11.	**Locusta migratoria* L.	»	»	
12.	**Trimerotropis latifasciata* SCUDD.	»	»	
13.	**Trimerotropis fallax* SAUSS.	»	»	
14.	**Trimerotropis suffusa?* SCUDD.	»	»	
15.	**Pseudotrimerotropis cyaneipennis* BRUNER	»	»	
16.	**Neopodismopsis ?abdominalis* THOMAS	»	»	
17.	**Tryxalis nasuta* L.	»	»	
18.	**Metapodius femoratus* FAB.	*Hemiptera*	*Heteroptera.*	*Coreidae*
19.	**Metapodius granulosus* DALL.	»	»	
20.	**Metapodius terminalis* DALL.	»	»	
21.	*Cimex lectularius* L.	»	»	*Cimicidae*
22.	*Banasa calva* SAY.	»	»	*Pentatomidae*

23. *Trichopepla semivittata* SAY.	*Hemiptera*	*Heteroptera*	*Pentatomidae*
24. *Aleurodes proletella* L.	»	*Homoptera*	*Aleurodidae*
25. **Nautococcus schraderae* VAYS.	»	»	*Coccidae*
26. *Blaps lusitanica* HERBST	*Coleoptera*	*Tenebrionidae*	
27. **Diabrotica 12-punctata* F.	»	*Crysomelidae*	
28. **Diabrotica soror* LEC.	»	»	
29. *Spermophagus (Zabrotes) subfasciatus* BOH.	»	*Bruchidae*	
30. *Ascalaphus libelluloides* SCHAEFF.	*Neuroptera*	*Myrmeleontidae*	
31. **Tipula paludosa* MEIG.	*Diptera*	*Tipulidae*	
32. *Drosophila calloptera* SCHINER (syn. *ornatipennis* WILLIST.)	»	*Drosophilidae*	
33. **Drosophila testacea* V. ROS (syn. *putrida* STURTEV.)	»	»	

Literature pertaining to the above list. — *1*: This paper. — *2*: MELANDER, unpubl. — *3*: STEVENS, 1912 a. — *4*: MAKINO, NIIYAMA and ASANA, 1938; WHITE, 1945, p. 119. — *5*: VOINOV, 1925; STEOPOE, 1939; WHITE, 1945, p. 179. — *6*: ROBERTSON, 1917; DARLINGTON, 1937, p. 145; WHITE, 1945, p. 119. — *7*: MCCLUNG, 1917; WHITE, 1945, pp. 251--252. — *8*: CARROL, 1920; DARLINGTON, 1937, p. 314; WHITE, 1945, p. 119. — *9*: CAROTHERES, 1917. — *10*: WHITE, 1949. — *11*: ITOH, 1934; DARLINGTON, 1937, p. 145; WHITE, 1945, p. 121. — *12*: WHITE, 1949. — *13—14*: CAROTHERES, 1917; WHITE, 1945, p. 112. — *15*: WHITE, 1949. — *16*: ROTHFELS, in the press; see WHITE, 1949. — *17*: MINOUCHI, 1934. — *18—20*: WILSON, 1907, 1909, 1910; DARLINGTON, 1937, pp. 142, 145, 313; WHITE, 1945, pp. 122, 259; SCHRADER, 1947. — *21*: SLACK, 1938; DARLINGTON, 1940; TROEDSSON, 1944; WHITE, 1945, pp. 249, 259, 265; SCHRADER, 1947. — *22*: WILSON, 1907; DARLINGTON, 1937, p. 145. — *23*: MONTGOMERY, 1906. — *24*: THOMSEN, 1927. — *25*: HUGHES-SCHRADER, 1942; WHITE, 1945, p. 119; SCHRADER, 1947. — *26*: NONIDEZ, 1921; DARLINGTON, 1937, pp. 145, 363; WHITE, 1945, p. 261. — *27—28*: STEVENS, 1908, 1912 b; HOY, 1914; WHITE, 1945, p. 119. — *29*: MINOUCHI, 1935. — *30*: NAVILLE and BEAUMONT, 1933; DARLINGTON, 1937, p. 58; WHITE, 1945, pp. 259, 265. — *31*: BAUER, 1931; WHITE, 1945, p. 245. — *32—33*: WHARTON, 1943; WHITE, 1945, p. 139.

III. SOME NOTES ON ANIMAL ACCESSORY CHROMOSOMES.

The above list includes several species in which supernumerary chromosomes are obviously like sex chromosomes, for instance as to the kind and degree of heteropycnosis and behaviour at meiosis.

In animals there are found transitions between sex chromosomes and accessory chromosomes. For instance, in observed populations of the common earwig in Europe, *Forficula auricularia*, the *X*-chromosome in part of the population can be found divided into two (according to WHITE's interpretation): thus one finds either one or two *X*-chromosomes in different individuals. In the orthoptera *Metapodius* the

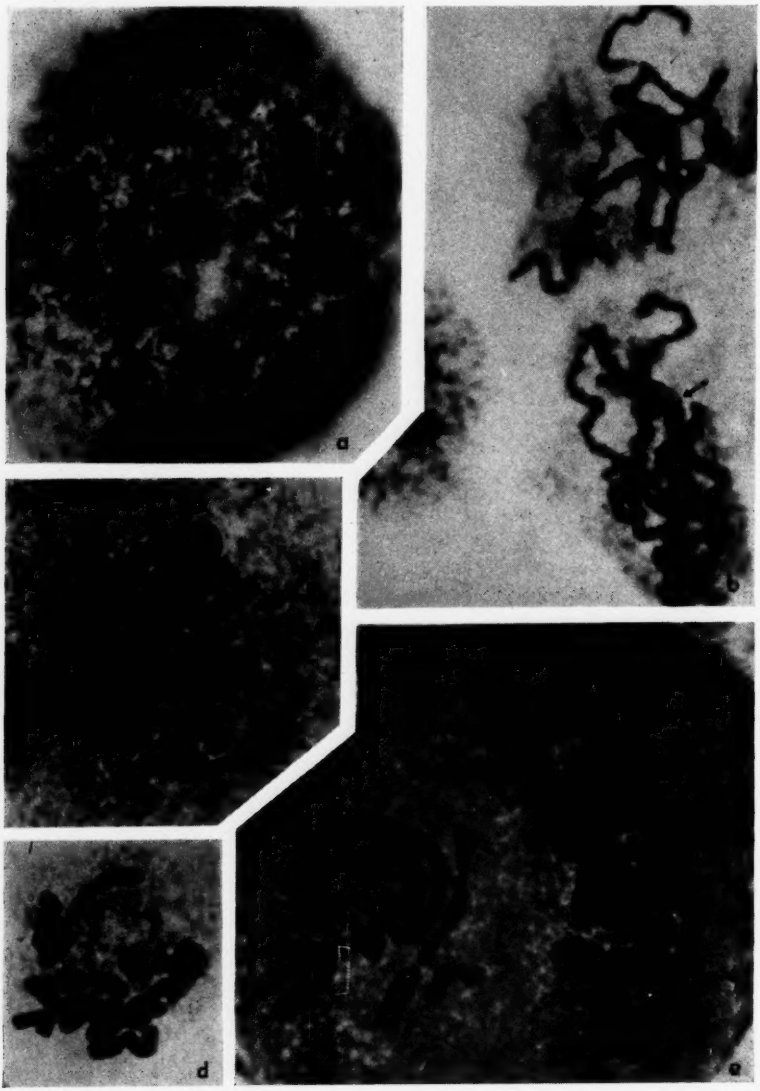

Fig. 1. — *a.* Nucleoli in the resting-stage of a blastomere nucleus. The blastomeres in this egg-cocoon contain 4 accessory chromosomes, all of which are able to form nucleoli. Together with these two other nucleoli are formed by the longest chromosome pair in the complement. — *b.* Late prophase of mitosis of a somatic cell from

accessory chromosomes vary in number but cannot be definitely distinguished from the *Y*-chromsome, and in the hemiptera *Cimex lectularius* the supernumerary chromosomes are most like *X* (possibly *Y*). In the neuroptera *Ascalaphus libelluloides*, too, the likeness of the supernumerary chromosomes to sex chromosomes is great, but it cannot be determined whether they are more closely related to *X* or to *Y* or possibly to both or neither.

In these cases the supernumerary chromosomes have probably arisen as derivatives of sex chromosomes and have not been eliminated, because, like the latter, they have been built up of material poor in genes. Their importance to sex determination has ceased, and they have increasingly developed their power of persisting and spreading in the population, facilitated by the fact that they, like most animal sex chromosomes, have aberrant centromeres. In some cases they have developed into real accessory chromosomes. Such autosomes as diverge in a similar way from the rest of the complement, *e. g.* the so-called microchromosomes in certain animals, may also be the origin of accessory chromosomes. In the hemiptera *Alydus calcaratus* there are two chromosomes (*M* and *m*), differing in size and aberrant from the other autosomes, which appear in varying combinations (*MM*, *Mm*, or *mm*) — REUTER (1930). There does not seem to be any reason, as far as I can see, to call them supernumeraries as DARLINGTON (1937, pp. 142 and 310) does, though they might possibly be considered a stage in the development towards accessory chromosomes.

In two parthenogenetic fresh-water triclads of the genus *Polycelis*, whose chromosome conditions will be described in a later paper, chromosome fragments are sometimes found that include only the centromere along with inconsiderable heteropycnotic parts of chromosome-arms on both sides of it (Fig. 1 *c*). It is possible that in certain cases fragments of this kind may form initial stages at the origin of accessory chromosomes.

The more often fragments arise that have a functioning kinetic

a full-grown animal containing 0—4 accessory chromosomes. Note that at this stage they do not differ in their degree of spiralization. The arrow points to the satellites of one of them. — *c*. Mitosis from another species of *Polycelis* with 21 chromosomes. In this case there are two kinds of fragments indicated by the arrows. The lower one consists merely of the centromere + small negatively heteropycnotic pieces of the chromosome-arms at both sides of it. — *d*. A »half» accessory chromosome consisting of the satellite-bearing arm of the normally metacentric accessory chromosome. Only this kind of accessory chromosomes were seen in this animal. — *e*. Mitotic metaphase of a blastomere nucleus with 4 accessory chromosomes. The arrow points to the satellites. — Stain: aceto-carmine. — × 2500.

mechanism, the more often we may expect to find fragments among them that are able to develop into accessory chromosomes. Fragments possessing their own power of motion arise with special frequency in animals with a so-called diffuse kinetochore (*cf.* HUGHES-SCHRADER and RIS, 1941). In his work on the bearing of the kinetochore on the origin of the »compound» chromosomes that frequently occur in these animals SCHRADER (1947) points out the importance of the diffuse kinetochore for their origin in *Hemiptera.* Within this group several instances (8 out of 33 in the previous list) are found of possible accessory chromosomes. There is reason to suppose that the diffuse kinetochore has contributed to the presence of both chromosome categories.

The prevalent notion that accessory chromosomes in plants, to be able to persist in the population, must be furnished with genes or other attributes that make them favourable for the individual, is also held by WHITE (1945, 1949) in regard to animals. ÖSTERGREN (1945), however, expresses a completely contrary opinion. He writes (p. 159): »The decisive point in the selection of fragments with a differential behaviour is how favourable their properties are *for their own existence.*» STEVENS (1908) occupies herself with the same thoughts when she describes the first indubitable accessory chromosomes in the coleopter *Diabrotica 12-punctata*; she assumes the possibility that the accessory chromosomes (»supernumeraries») arose before the two species separated and that, on account of their behaviour, they have in the course of time attained the same distribution in them. Moreover, it is evident that, in several cases, similar accessory chromosomes are found in closely related species (*e. g., Gryllotalpa gryllotalpa—africana, Trimerotropis latifasciata—fallax—suffusa, Metapodius femoratus—granulosus—terminalis* and *Diabrotica soror—12-punctata*). This may be taken to show that the accessory chromosomes of related species have arisen in a common initial form, but it may also be explained as a result of recent hybridization. The fact that undoubtedly they are sometimes harmful to the organism speaks in favour of ÖSTERGREN's opinion that the accessory chromosomes have their own line of development. These injurious effects are particularly evident in *Polycelis tenuis.* Such effects are also found in the crane fly, *Tipula paludosa,* according to BAUER (1931), who writes that the presence of accessory chromosomes causes harm by strongly disturbing the spermatogenesis. In most cases among animals, however, it is not known what influence the accessory chromosomes have. It ought to be pointed out as important for this discussion that the value of accessory chromosomes

to an organism should be judged when they are functioning in the biotope of the population and not under changed conditions.

The power of accessory chromosomes to maintain a numerical balance within the population depends on two main circumstances. Firstly, they must not have too unfavourable an effect on the individual in whose nuclei they are; in other words they must be fairly indifferent. The other condition is that their action must be such as to regulate their number. These conditions are brought about in the accessory chromosomes of many species according to the following survey:

(1) Relatively little influence of the accessory chromosomes on the organism
   (a) owing to the occurrence of much heterochromatin in them (in itself poor in genes); this most often causes them to show *allocycly with heteropycnosis* during some part of the nuclear cycle;
   (b) owing to the occurrence of genetically inert euchromatin.

(2) A behaviour differing from the other chromosomes with multiplication or elimination as a result
   (a) owing to aberrant centromeres; this often brings about: (α) *elimination* if the centromere is kinetically weak, (β) *multiplication* if it divides more often than that of the ordinary chromosomes, and (γ) the appearing of iso-chromosomes or telocentric chromosome-arms if it tends to mis-division, which thus causes *variation in size* if the original chromosome was not metacentric;
   (b) owing to diverse behaviour of different parts of the chromatids; this causes *multiplication* that may be due to divergences in the synapsis of meiosis or differing cleaving conditions at certain mitotic divisions (*e. g.* pollen mitosis in plants).

Thus accessory chromosomes form a heterogeneous category. Common characteristics, however, are varying number and non-homology compared with ordinary chromosomes of the species.

IV. ACCESSORY CHROMOSOMES IN POLYCELIS TENUIS IJIMA.

In a population of the fresh-water tricladide turbellaria *Polycelis tenuis* IJIMA from the lake Vombsjön in Scania, Sweden, some of the worms turned out to have accessory chromosomes. Since nothing was known earlier about these in other animals than insects, I thought it important to study the frequency in which they occurred in the population as well as their behaviour and possible influence on the worms.

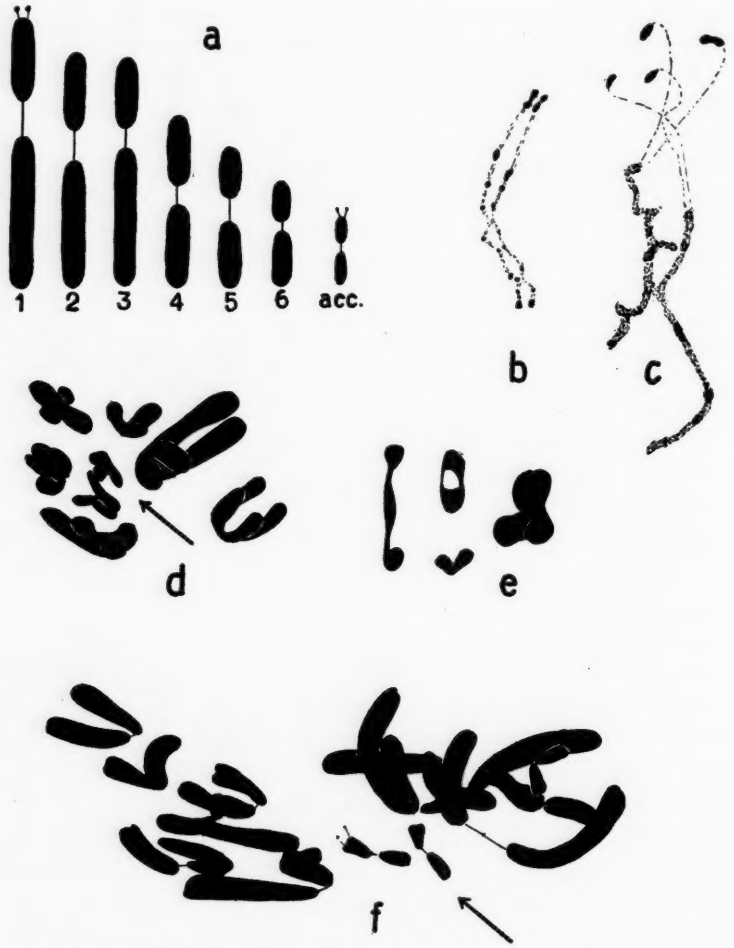

Fig. 2. — *a.* Scheme of the mitotic chromosomes of *Polycelis tenuis* with accessory chromosomes. — *b, c.* The accessory chromosomes in the prophase of meiosis in testis and ovary respectively. Note the long thread without chromomeres, which has a knob distally. *Cf.* Fig. 3 *c.* — *d.* II-M of the spermatogenesis with two accessory chromosomes indicated by an arrow. — *e.* Different configurations of accessory chromosomes at I-M during spermatogenesis. — *f.* Mitotic metaphase in a somatic cell from an animal with 0—4 accessory chromosomes. The arrow points to two of them. Note that their diameter is smaller at this stage. — Stain: aceto-carmine. — × 4000.

Hence some hundreds of full-grown individuals of the worm were collected from the lake and isolated for later study. They were kept at about 18° C. It turned out, however, that the presence of accessory chromosomes affected the animals badly under the circumstances in which they were grown, so that in some cases they died, and for that reason no reliable figures could be obtained in that way of the frequency of accessory-chromosome animals. It also turned out that in practice the presence of these chromosomes could be determined only in testicular tissue at meiosis, because, as a rule, they had been eliminated from the somatic cells. I found there could be only one way in which to determine their frequency, *viz.* to fix parts of the sexually mature animals immediately after collecting them for chromosome study (made according to MELANDER, 1948) and to keep the rest alive for regeneration at a low temperature (7° C) in case other investigations or crossings were desired. Nor are the figures — obtained in this way from material collected on a single occasion — reliable as to the frequency of accessory-chromosome animals within the population. This is further discussed below. A figure of 20 % is mentioned only to give an idea of how frequent the accessory chromosomes are in *Polycelis tenuis* in the lake Vombsjön.

1. MITOSIS.

The number of ordinary chromosomes in *Polycelis tenuis* is $2n=12$.

The accessory chromosomes are smaller than any of the others. Their length at the metaphase of mitosis in somatic cells of full-grown animals does not reach 2 μ (Fig. 2 *a*, *f*). It is to be noticed that their breadth is only half of that of the others. They are furnished with a median centromere and satellites, similar to those on the longest chromosome of the complement (No. 1, Fig. 2 *a*; see also Fig. 1 *b*, *e*). At the metaphase of mitosis they are stained with aceto-carmine to about the same extent as the others, yet they are, in certain cases, evidently negatively heteropycnotic at this stage (Fig. 3 *a*), and sometimes their outlines are not quite so even as those of the other chromosomes. There is no difference in general appearance between accessory chromosomes and ordinary chromosomes during mitosis of the early blastomeres and during prophase of older cells. In both cases all the chromosomes have a short spiral diameter and the matrix is slightly developed (Fig. 1 *b*, *e*).

Besides the normal type of accessory chromosomes described above, chromosomes have been observed that evidently consist merely of the satellite-bearing arm of the normal type of accessory chromosome and

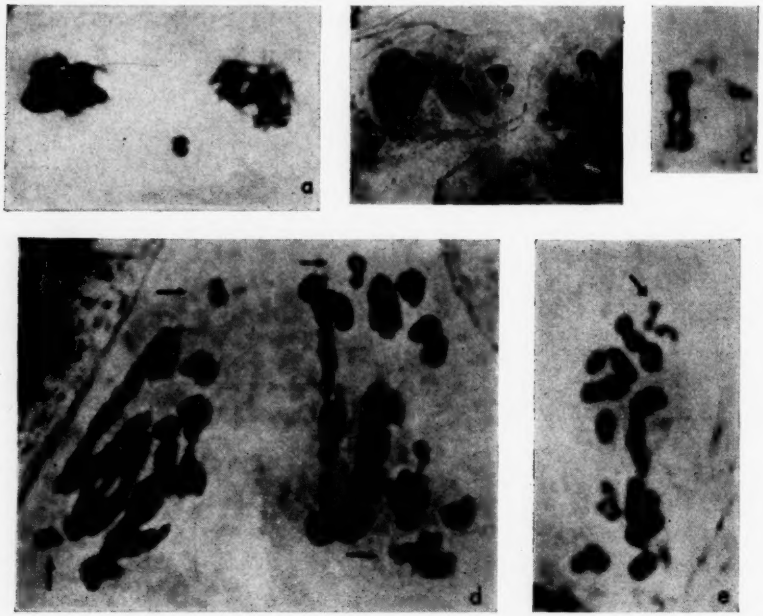

Fig. 3. — *a*. Telophase of the second meiosis during spermatogenesis of an animal with accessory chromosomes. In this case one of these has been expelled from the nucleus. — *b*. Spermatids with adjoining chromatin granules consisting of accessory chromosomes; × 830. — *c*. Accessory chromosome in the prophase of the ovary meiosis (*cf*. Fig. 2 *c*). — *d*. Anaphases of 1st meiosis during spermatogenesis of an animal with 0—4 accessory chromosomes. — *e*. 2nd meiosis with accessory chromosomes. Note that the last-mentioned are negatively heteropycnotic at this stage and that hey have a small diameter. — Stain: aceto-carmine. — × 2500 (except *b*).

centromere (Fig. 1 *d*) and, in one case, of the second, non-satellite-bearing arm of the normal type. Accessory chromosomes of the same type are present in the whole animal. I have not been able to determine whether the centromere in these accessory chromosome-arms is situated entirely terminally or whether, possibly, a small part of the other arm lies outside it.

The accessory chromosomes occur by no means in all the cells of the animal but are missing in most cells of fairly old and full-grown animals. The early blastomeres of the egg-cocoon, which all contain the same number, form an exception. The distribution of accessory chromosomes in two hatching embryos from the same egg-cocoon is evident from Table 1.

TABLE 1. *Distribution of the number of accessory chromosomes at mitosis from two sister-embryos. (A = members of the ordinary complement.)*

Contents of plates:	12 A + 0 acc.	12 A + 1 acc.	12 A + 2 acc.	12 A + 3 acc.	12 A + 4 acc.
Number of plates:	30	3	5	0	3

The distribution of accessory chromosomes in somatic cells from full-grown animals corresponds to that in older embryos. Possibly they are still rarer in the former.

Such mitotic divisions as those in which the elimination of the accessory chromosomes has been directly observed have been found only in the blastomeres of the egg-cocoon. Here chromatin grains occasionally appear between two telophase-nuclei, indicating that the accessory chromosomes are not always transported to the poles at anaphase but remain between the daughter nuclei and so disappear. Those cells of *Polycelis tenuis* which have lost their accessory chromosomes probably do better service within the organism than those which still keep them. If that is the case, the former are likely to gain ground gradually and in somatic tissues replace those which are afflicted with the accessory chromosomes. The fact that the latter are found regularly in the gonads probably signifies that the undifferentiated, amœboid, blastomere-like cells occurring in the *Turbellaria* are not disturbed by the presence of accessory chromosomes; as before mentioned, no elimination of accessory chromosomes takes place in the blastomeres. I am inclined to assume that the germ track — the connection between the zygote and the gametes from the gonads that will develop out of it — is localized to the above-mentioned undifferentiated cells.

2. MEIOSIS.

In testes the number of accessory chromosomes varies from one cell to another, but, as already said, they occur there in larger number than in somatic cells. Still, I never saw cells in the gonads with more than 4 accessory chromosomes. In a single egg-cocoon, however, I found blastomeres, containing 8 of them. At I-M they appear in testes as univalents or bi-, tri- or tetra-valents. Table 2 illustrates the condition in 77 nuclei at I-M in testes of one animal.

Of the 34 bivalents in Table 2, 23 were provided with one and 11 with two chiasmata. The chiasmata of the accessory chromosomes, as far as I can see, are always terminalized at metaphase. As the ter-

TABLE 2. *The distribution of 77 cells with accessory chromosomes with regard to number and configuration at I-M in testes of one specimen of Polycelis tenuis.*

Number of acc. chr.:	0	1	2		3			4			
Configuration of acc. chr.:	—	1_I	2_I	1_{II}	3_I	$1_{II}+1_I$	1_{III}	4_I	$1_{II}+2_I$	$1_{III}+1_I$	1_{IV}
Number of cells in each category:	9	16	4	26	2	6	7	0	2	3	2
	9	16	30		15			7			

minalization is complete in the metacentric accessory chromosomes, the bivalents get the appearance indicated by Fig. 2 *e*. The centromeres of the accessory chromosomes divide a little before those of the other chromosomes and they reach the poles first at anaphase (Fig. 3 *d*).

At II-M the accessory chromosomes do not diverge in their general appearance except by their inconsiderable size (Fig. 2 *d*). In certain cases, however, they are evidently negatively heteropycnotic. In the specimen recorded in Table 2, cells with 0—3 accessory chromosomes in II-M of testes were distributed in the following way:

TABLE 3. *Number of accessory chromosomes in 100 cells at II-M in testes of one specimen of Polycelis tenuis.*

Number of acc. chr.	0	1	2	3
Number of cells	27	51	22	0

This distribution does not diverge from expectation, to judge by the distribution of the accessory chromosomes at I-M, which implies that no loss of accessory chromosomes worth mentioning between I-M and II-M can be assumed. On the other hand, many accessory chromosomes vanish during the anaphase of the second meiotic division, which is inferred from the fact that small chromatin grains are found between both anaphase- or telophase-groups and also near the spermatids (Fig. 3 *a*, *b*). Thus, out of 500 spermatids, 86 occurred with adjoining grains of such size that they could be supposed to contain accessory chromosomes. (In addition there appeared 5 spermatids with a rather large adjoining chromatid-piece that was probably derived from some other eliminated chromosome, possibly exceptional univalents that had lagged in the first meiotic division and had been expelled from the nuclei.) It is to be supposed that this lagging and elimination during the anaphase of the second meiotic division is connected with differences

between the centromeres of the accessory chromosomes and those of the other chromosomes.

As to the ovarial meiosis, it has only been possible to study the prophase in the animals with accessory chromosomes. It turned out, however, that the accessory chromosomes, when first observed, always lie in pairs close together and consequently appear in even numbers. Thus nuclei with only one accessory chromosome have not been observed at the ovarial meiosis, nor nuclei in which the accessory chromosomes have not paired. (It has been possible to analyse about 20 nuclei in this respect with sufficient degree of reliability.) I have interpreted the above-described conditions to indicate that the accessory chromosomes, contrary to the others, pass through an endomitotic duplication in the resting nucleus that precedes I-M. This is also the case with all the chromosomes of the complement of two *Polycelis* species, whose chromosome conditions I intend to describe in a later paper.

In the prophase (pachytene) of meiosis the accessory chromosomes have the appearance shown in Fig. 2 *b*, *c*. Particularly notable is a long, extremely thin section, situated at the end of the chromosome, which is provided with one big distal chromomere. This section is evidently the same as forms the satellite-neck at the mitotic metaphase. A close study of the appearance of the remaining chromosomes at pachytene has not been made, but in members of the ordinary complement I have failed to find any such chromomere constellations as are found in the accessory chromosomes. In view of the presence of satellites on the accessory chromosomes, one would expect them to be homologous with the proximal part of chromosome No. 1, which is also provided with satellites. That, however, is certainly not the case. Nor have I ever seen any pairing between accessory chromosomes and ordinary chromosomes.

3. RESTING STAGE.

During the resting stage the accessory chromosomes cannot be observed, as might have been expected, since they are heteropycnotic at certain stages.

They form a small nucleolus, at least in blastomere nuclei (Fig. 1 *a*). In the early blastomere resting-nuclei of an animal with 4 accessory chromosomes there are 1—6 nucleoli, while animals that lack accessory chromosomes have 1—2 nucleoli in these nuclei.

4. THE INFLUENCE OF THE ACCESSORY CHROMOSOMES ON THE ORGANISM.

A specimen containing 0—4 accessory chromosomes was crossed with another of identically the same kind. This was rendered possible after two clone-animals had been produced by making pieces of the original animal regenerate into two new individuals, which became sexually mature. As these worms are hermaphrodites, the clone-animals could be crossed with each other. To begin with, however, it was not possible to make them copulate. So the first egg-cocoons produced by the animals did not contain any embryos. However, they copulated at last, and a fairly small I_1 could be obtained. In Table 4 the result is shown of this strict inbreeding in animals with accessory chromosomes, and this is compared with the average result from several similar crossings of animals without accessory chromosomes, carried out on material, also taken from the lake Vombsjön and treated in the same way.

It took more than one month — as compared with about a fortnight ordinarily — before the egg-cocoons hatched. The progeny was also less than normally. (A retarded development is the consequence in normal animals as well if the cocoons contain few embryos, but the development goes on more rapidly in the latter case.) The temperature was kept at 23° C throughout the experimental period.

TABLE 4. *Result of inter-clone crossings in Polycelis tenuis from the lake Vombsjön, partly with accessory chromosomes in the clone, partly without them. Temperature of culture 23° C.*

	Number of cocoons	Number of I_1-animals	Number of surviving I_1-animals
After self-fertilization of animals with acc. chr.	10	9	2
After self-fertilization of animals without acc. chr.	10	60—70	60—70

Now we see that strict inbreeding of worms having accessory chromosomes gives a particularly bad result as compared with that of animals lacking them. It is evident that only a reduced number of embryos hatch, and that the mortality among them is great when they are cultured in this way.

All the I_1-animals with accessory chromosomes that died did so under similar symptoms of disease. On the ciliated epithelium wounds arose through which mesenchyme cells emigrated, so that the worms assumed a monstrous appearance and generally died (Fig. 5 *d*, *e*). Old

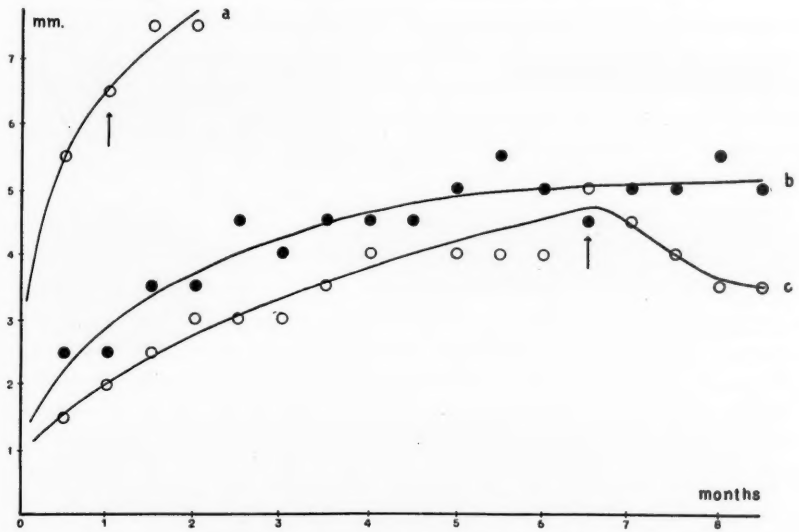

Fig. 4. — Diagram showing the growth of I_1-animals. — *a*, without accessory chromosomes, *b*, *c*, with 0—4 accessory chromosomes in their cells. The arrows indicate the time it was possible to see the copulatory organs. At the top of the curve *c*, the animal was affected by the disease described in the text, which accounts for the drop of the curve. Temperature of culture 23° C. *Cf.* Fig. 5.

animals, too, can get the same disease. They sometimes survive and then gradually regenerate the destroyed parts of their bodies. If the animals are kept at a low temperature, however, no such injuries arise, which is probably due to the greater proportion of oxygen in water at a low temperature. This is indicated by the circumstance that worms with accessory chromosomes, kept in culture at 23° C, spend most of their time hanging on the surface, which is not the case with animals without these chromosomes, living under the same conditions, or with those having them, but kept at a low temperature.

The length of two I_1-specimens from the above-mentioned inbreeding of animals, containing accessory chromosomes, has been measured fortnightly together with a control-animal from a similar cross in which the parents lacked the accessory chromosomes. The temperature was here 23° C throughout the experiment. The control-animal grew in the same way as many other similar worms, whose length, however, was not measured. The results of these measurements are brought together in the diagram Fig. 4, and the three worms were figured at the time the

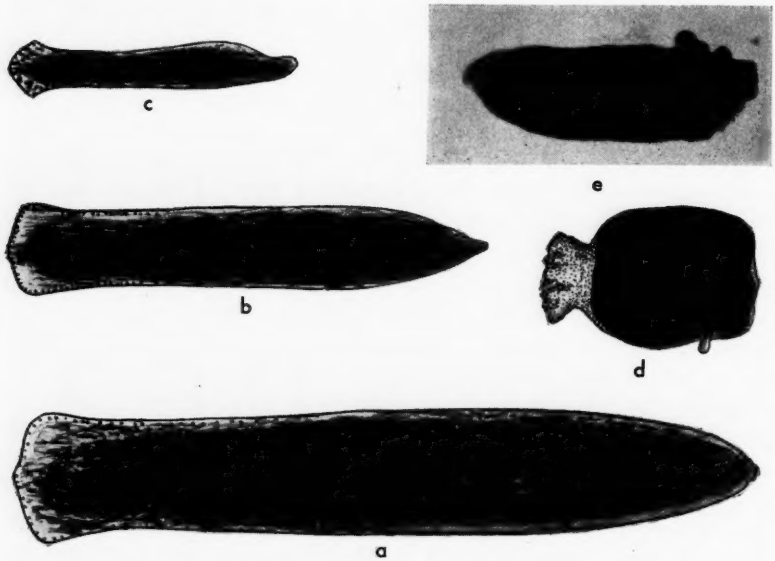

Fig. 5. — *a, b, c.* The animals of Fig. 4 at the moment when the measurements were finished; × 7,5. — *d, e.* Embryos, a few days old, containing 0—4 accessory chromosomes and attacked by the disease mentioned in the text. — × 30.

measurements were finished (Fig. 5 *a, b, c*). It appears from the measures that the two animals with accessory chromosomes have grown extremely slowly. The smaller of the two sister-animals was attacked by the disease described above, which accounts for the irregular course of the curve in its latter part. The development up to the time the organs of copulation began to appear took about six months, as against normally one.

The I_1-worms suffer greater damage from their accessory chromosomes than animals taken in nature and provided with these chromosomes; this in spite of the fact that the accessory chromosomes are found in equal number in both groups (0—4 in both, with at least approximately the same distribution within the individual). This indicates the possibility that in the ordinary chromosomes there are genes affecting the organism unfavourably in the homozygous state. Another possible explanation is that inbreeding depression will not appear very distinctly in I_1 if the worms are not troubled with the presence of the accessory chromosomes.

As I have mentioned before, animals with accessory chromosomes thrive obviously worse under ordinary laboratory conditions than normal animals do. Of course, the oxygen content in the littoral zone of a lake is much higher than in a glass-tube with stagnant water, which has a small surface to the air. Should the accessory chromosomes essentially reduce the vitality of the organism under natural conditions as well, they will doubtless soon disappear from the population.

On account of their slow development, specimens provided with accessory chromosomes do not become sexually mature until late summer or autumn, when most other animals of the species have already finished their reproductive activities. The proportion of sexually mature animals with accessory chromosomes will therefore be greatest towards the end of the hot season. The population within which the crossing of accessory-chromosome animals is possible becomes reduced in number. As a consequence such animals obtain more opportunities to copulate with one another, and the number of accessory chromosomes in the progeny will increase when gametes containing them conjugate. Further, crosses between animals possessing accessory chromosomes and animals not possessing such will introduce the accessory chromosomes into animals with a disposition for late sexual maturity, which must be a property of both parents. The combination of retardment, caused by accessory chromosomes and hereditary disposition, is not favourable to the animals if it makes sexual reproduction more difficult when the water in which they live is getting colder. Facts are as yet lacking concerning this question. It is evident, however, that the accessory chromosomes bring about physiological isolation within the population by allowing sexual maturity only towards the end of the reproduction period of the species.

SUMMARY.

It is proposed that the term *accessory chromosome* should be generally adopted to denote such extra chromosomes as are not homologous with those of the ordinary complement and whose number is in equilibrium in a population.

A list of animal species with accessory chromosomes is given together with a brief discussion of the properties of accessory chromosomes.

In the fresh-water triclade turbellaria *Polycelis tenuis* IJIMA accessory chromosomes were found in one population:

(1) Their number is balanced in the population, and they occur in about 20 % of the worms to a number of 0—4 (8) in each cell. Exceptionally one finds only one of the two chromosome-arms in an animal.

(2) Accessory chromosomes have a centromere that differs in strength and division-rhythm from that of the other chromosomes. The result of this is a multiplication in the ovarial meiosis and an elimination from somatic cells.

(3) They are provided with satellites and intervene in the nucleic-acid metabolism by forming nucleoli.

(4) They are weakly but clearly heteropycnotic.

(5) They affect the organism detrimentally under certain environmental conditions, and, owing to the aberrant physiology of the accessory-chromosome animals, such crosses in which they appear can only occur within a limited part of the population in nature. This increases the opportunities of multiplication of the accessory chromosomes by means of new gametic combinations.

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SPONTANEOUS CHROMOSOME VARIATION IN THE ROOTS OF A SPECIES HYBRID

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INTRODUCTION.

THE observations to be described here were made in root tips of hybrids *Godetia nutans* ($n = 14$) ♀ × *G. Whitneyi* ($n = 7$) ♂. *G. nutans* is a rather variable species that has been described by Dr. GUNNAR HIORTH (1941, 1942). I have investigated several hybrids between different subspecies and races of *G. nutans* on the one side and *G. Whitneyi* on the other. The chromosome pairing of these hybrids is remarkably constant; 7 II + 7 I was always seen in the p.m.c.'s (HÅKANSSON, 1942). Thus, *G. nutans* seems to be an allo-tetraploid with one genome from *G. Whitneyi* and one unknown genome. At least one race of *G. nutans* has accessory chromosomes: plants from Santa Rosa, California, often have more than 28 chromosomes. The meiotic pairing of the supernumeraries is strange: plants with 29 chromosomes always form 14 II + I, some plants with 30 chromosomes form 15 II or less often 14 II + 2 I, other plants 14 II + 2 I. Meiosis has also been studied in 31- and 32-chromosome plants, and the result tends to show that different *nutans* supernumeraries have different pairing properties (HÅKANSSON, 1945). Only in one hybrid plant *G. nutans* S. Rosa × *G. Whitneyi* was meiosis studied: the pairing 7 II + 8 I was found, confirming the inability of the supernumerary to pair with ordinary chromosomes (HÅKANSSON, 1942). *G. nutans* plants from the locality Magalia also had more than 28 chromosomes, but this case has not been studied further. Thus, while it has been shown that the supernumeraries of S. Rosa have some of the properties of accessory chromosomes or B chromosomes it is unknown whether the supernumeraries of Magalia possess such properties.

The material of this investigation is F_1 hybrids between *G. nutans* S. Rosa with accessory chromosomes and *G. Whitneyi*. A *nutans* plant having the pairing 14 II + 2 I (1281/954—1943) had been selfed, and I had studied meiosis in some plants of the offspring. One plant had

14 II + I, one had 15 II, one had 14 II + 2 I; the two supernumeraries of the mother plant thus seemed to be different. These sister plants had been pollinated from the same plant of *G. Whitneyi*. Dr. HIORTH made the crosses in order to transfer accessory *nutans* chromosomes to a diploid species. Seeds from these crosses were also sown at Svalöf to determine the chromosome numbers of F_1 plants. They ought to have 21 A chromosomes and perhaps one or more accessory chromosomes. Only root tips have been fixed and meiosis has not been studied. The F_1 hybrids are very sterile. HIORTH (1942) writes that the many species hybrids »zwischen den verschiedensten Typen von *nutans* und *Whitneyi* stets eine sehr schlechte Samenfertilität haben (nur vereinzelne Samen je isolierten Zweig)»; this is the reason why no offspring have been procured from these plants.

The aim of the cytological investigation was to try to show differences between the crosses in the frequency of plants with B chromosomes. However, unexpected difficulties arose in determining the chromosome numbers of some plants. Most plants showed a numerical variation. Root tips from the same plant often had different chromosome numbers — interradicular variation, and in many roots the chromosome number varied — intraradicular variation. Such frequent variations in chromosome number are rare and therefore all root tips that had been fixed were subjected to a close investigation. As a comparison *G. nutans* from S. Rosa was also studied. These seeds had also been procured from Dr. HIORTH.

GODETIA NUTANS S. ROSA.

Previous investigations had not shown any oscillation in chromosome number within the same plant. In most cases six roots of each plant were studied closely. Three plants had 28 chromosomes, four had 29, and two had 30. No intraradicular variation could be observed, and interradicular variations also seemed to be absent. From this constancy of chromosome number, however, one exception was observed. From one plant 10 roots had been fixed; nine of them had 29, while one had 28 chromosomes. This exception may be due to an error at the fixing or imbedding of the roots. No disturbed mitosis was observed in the roots. It accordingly seems as though chromosome variations are absent or very rare in *G. nutans* as well as in such plants as have supernumerary chromosomes. This contrasts with the behaviour of the root chromosomes of the triploid species hybrids that were grown at the same time.

GODETIA NUTANS S. ROSA × G. WHITNEYI.

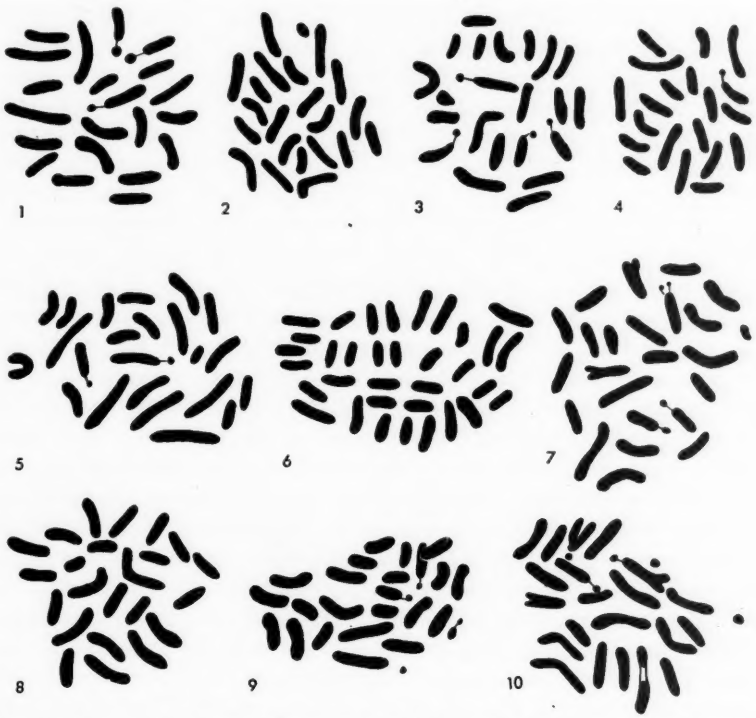
Only a minority of the plants had a constant chromosome number. The number of clear plates is of course different in different roots, but 20—40 or more could be studied. The number of roots fixed was also different. A close analysis of the plates could not be made owing to the fact that there could not be seen any constriction or unstained section at the centromere region of the chromosomes. As a rule there were three satellite chromosomes (Fig. 1). Two of them were short and no doubt morphologically identical; rather often their satellites were quite near one another in the plate, probably because they were in connection with the nucleolus in the preceding prophase. One of these small satellite chromosomes is a *Whitneyi*-, the other, a *nutans*-chromosome. The third satellite chromosome is larger and belongs no doubt to the foreign genome of *G. nutans*. More rarely one observes four satellite chromosomes, the fourth doubtless being a *Whitneyi* chromosome (compare HÅKANSSON, 1942). Three chromosomes are larger than the rest. It was not possible to distinguish the accessory chromosome from the A chromosomes.

14 II + I × 7 II. — From this cross 18 plants were investigated. The expected chromosome numbers were 21 and 22. Only five plants seemed to have a constant number, all plates observed having 21 chromosomes. Only 3 roots were fixed of one of these plants, but in the other cases 9 roots could be studied. A number of plants had a small intraradicular or interradicular variation. Such was *8—2*: 6 roots had apparently only 21-chromosome plates, 2 roots had 21-chromosome plates, but also a few with 22 chromosomes, but one root was more deviating. The last-mentioned had 21 chromosomes and a small fragment (Fig. 2). This fragment chromosome was clearly seen in many plates but could not always be observed, probably sometimes being lost during mitosis; once 22 + fr. was seen. Plant *8—15* had 21-chromosome roots, but sometimes one of them also had a larger fragment, and two some 22-chromosome plates. Plant *8—7* had 21 chromosomes as a rule but in two of nine investigated roots a few 22-chromosome plates occurred. A similar variation was disclosed by *8—16*. Relatively small was also the variation in *8—13*, where the plates in 7 roots always showed 22 chromosomes while two roots were deviating. One of them had 23 chromosomes, the other had a chimærical structure; one half of the root had 22 chromosomes, while in the plates of the other half there were 22 chromosomes and a small fragment chromosome. The latter seemed

to be somewhat larger than the fragment in *8—2* (Fig. 3). A rather strong variation was shown by *8—5*. This plant probably had 21 chromosomes (most roots investigated had this chromosome number, with perhaps some 22-chromosome plates). But here, also, a fragment root was found, the fragment chromosome being relatively small; plates with 21 + fr. and with 22 + fr. were found (Fig. 5). One root had only 20 chromosomes (Fig. 4); this number was observed in many plates (only one plate with 21 chromosomes was found). It was the only hypotriploid root in this investigation. *8—11* had a smaller variation: in six roots all plates observed had 22 chromosomes, while in one most plates had 22 chromosomes, though a few had 23; a further root showed in some plates a fragment, but it is uncertain whether it was a centric one.

The plants now described may show chromosomal variation but it seems rather clear that 10 of them came from a fertilized egg-cell with 21 chromosomes while only 2 were from a cell with 22 chromosomes. In the remaining six plants the chromosome number varied more frequently, and it was rather difficult to guess the true chromosome number. However, since plants with 21 chromosomes as a rule show a less frequent variation than plants with 22 chromosomes, it may be assumed that the egg-cells had had 22 chromosomes. Thus the cross had resulted in 10 plants lacking a B chromosome and 8 plants with one B chromosome. Now, the observations of meiosis in the p. m. c.'s have shown that the B chromosome, in spite of being unpaired, is in most cells not eliminated during the divisions. The result of this cross accordingly seems to be what would be expected from the behaviour of the B chromosome at meiosis.

The chromosome variation observed in these uncertain plants may be briefly described. *8—3* had five roots with 22 chromosomes, and three roots with 21, while in one root both chromosome numbers were often observed. One of the 22-chromosome roots had some plates with 23 chromosomes. *8—4* also had roots with 22, with 21, and with both these numbers. *8—8* was very variable. Several roots had 21 and 22 chromosomes, and in one of them a plate with 34 chromosomes was observed (Fig. 6). How this chromosome number has arisen is not easy to understand. One root with 22 chromosomes had a deviating plate with 23 and a very small, perhaps centric, fragment (Fig. 7). In one root there was a larger fragment chromosome, though it was not found in all plates (Fig. 8). *8—10* had roots with 21, with 22, and with both these numbers. Here in one root several plates seemed to have a very small fragment. The fragment was somewhat larger than a satel-



Figs. 1—10. — *1*: 21 chromosomes. — *2*: 21 + fr. — *3*: 21 + fr. — *4*: 20 chromosomes. — *5*: 22 + fr. — *6*: 34 chromosomes. — *7*: 23 + fr. — *8*: 21 + fr. — *9*: 23 + fr. — *10*: 21 + 2 fr?

lite and perhaps was a satellite, though this could not be decided. A 23-chromosome plate was also seen in this root (Fig. 9). *8—14* had two roots with 24 chromosomes, one with 23 and 24, one with 23, two with 22 and 23, three with 22, one with 22 and 21 chromosomes. Fig. 10 shows a rather strange plate with two fragments, probably acentric and similar to satellites. *8—18* had 22 chromosomes in four roots, 21 and 22 in three roots, and 21 in four roots. Here was found the only plate with a doubled chromosome number that has been observed in this investigation.

15 II × *7 II*. — The mother plant had 30 chromosomes, and as the supernumeraries mostly formed a bivalent, one could expect plants with 22 chromosomes in this cross. 19 plants were investigated closely. With one exception none of them had a quite constant chromosome

number, though some had only few deviating plates. A number of plants showed a more or less pronounced interradicular or intraradicular variation. The plant lacking variation had 22 chromosomes. Six or seven plants were fairly constant. One of them (*7—19*) showed 21 chromosomes in five roots, while in one root a few 22-chromosome plates were also found. Five of this fairly constant group of plants were 22-chromosomal. In most of them some plates with 23 chromosomes were also observed, in one plant a plate with 24 chromosomes was seen. To this group may also be assigned the plant *7—20* having 23 chromosomes in five roots and 22 chromosomes in one root. In this case it seems probable that the fertilized egg-cell had had 23 chromosomes.

The original chromosome number of the more variable plants was in most cases 22. Solely one plant (*7—11*) could be suspected to be 21-chromosomal, for only one root had 22 chromosomes while five had 21 (with some deviating plates having 22 chromosomes). The other plants (11) were mostly 22-chromosomal, most root tips having this chromosome number. A few of them also had some roots with 21 chromosomes. In most roots with 22 chromosomes a plate with 21 or 23 could be encountered. Roots with 22 and 23 chromosomes or whole roots with 23 chromosomes were also present. More rare were plates or whole roots having 24 chromosomes (Fig. 11). The variability in some plants was interesting. Plant *7—16* had roots with 22 chromosomes. One root, however, was more interesting because it was a chimæra, plates of one (longitudinal) half of the root having 22 chromosomes, of the other 23 (with some 22 plates). The root initials must in this case have had different chromosome numbers. The chromosome number of the epidermis is unknown. Another interesting root was presented by plant *7—6*. Here only few roots were investigated: one had 22 chromosomes, one had 22 or more rarely 23. A third root had 22 chromosomes and a rather large fragment chromosome. No plate lacking a fragment was observed, and one or two plates had two fragments (Fig. 12). It is uncertain whether this variation in number is due to non-disjunction or to other causes. *7—4*: in a root with 22 chromosomes a single plate with 23 was observed, the new chromosome was smaller than any of the ordinary chromosomes. Strong variation was shown by *7—17* with 21 (one root), 21 and 22 (one root), 22 and 23 (one root), 21, 22 and 23 (one root) chromosomes. One of the investigated roots from *7—12* had 21—24 chromosomes. *7—9* had five roots with 22, three had 23, and two had 23 and 24 chromosomes.

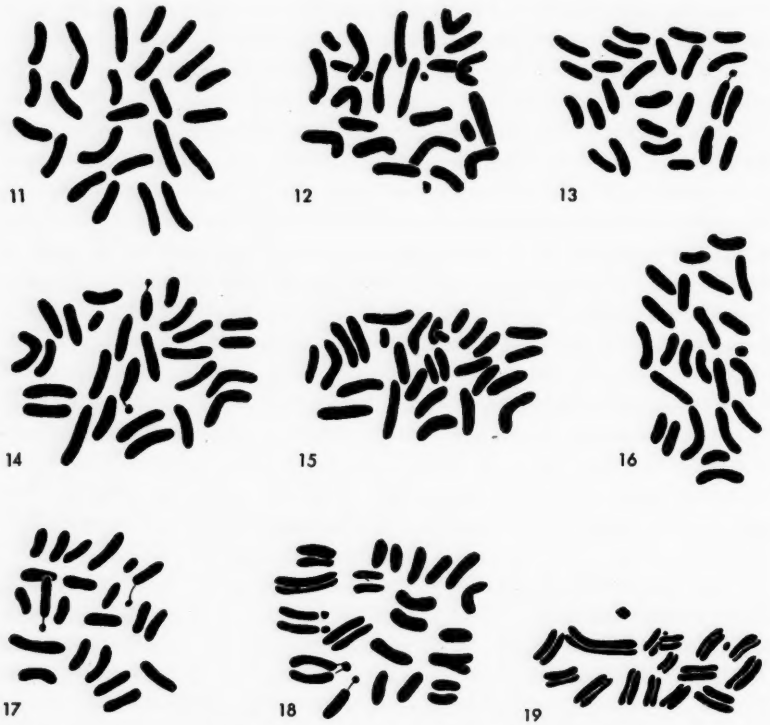
It seems that in this cross at least 15 but possibly 17 plants had

22 chromosomes while one or two had 21, and one or perhaps two 23 chromosomes. This seems to be a result such as one could expect from the behaviour of the two B chromosomes of the mother plant at meiosis.

14 II + 2 *I* × 7 *II*. — In this cross, too, the mother plant had 30 chromosomes but the supernumeraries were unpaired. The univalents are not subject to more frequent elimination at meiosis, but their distribution at the first anaphase must result in the embryo-sacs having 0, one or two supernumeraries. One expects plants with 21, 22 and 23 chromosomes in this cross and a preponderance of 22-chromosome plants. The number of investigated plants was 20. The number of 21-chromosome plants was definitely larger than in the preceding cross. In three plants all plates observed had 21 chromosomes, the number of roots investigated being 11, 6 and 4. One plant had 21 chromosomes in nine roots, but one of the roots had one or two plates with 22 chromosomes, and in another root a small group of cells had 22 (plates being found in several adjacent cells). The plant *6—1* had 21 chromosomes in five investigated roots, and 22 in two roots. Thus, four or possibly five plants may be regarded as 21-chromosomal.

No other plant than the three just mentioned had a constant chromosome number. This confirms the impression that plants lacking supernumeraries are less variable than plants with supernumeraries. The true chromosome number was in several cases uncertain. Three plants very probably came from a fertilized egg-cell with 23 chromosomes. *6—15* had 23 chromosomes in 12 roots; this number would have been called constant if a plate with 24 chromosomes had not been observed on one occasion; *6—17* had 23 chromosomes in five roots, 24 in two roots (in one of them were also a few 23-chromosome plates) and 22 in one root; *6—10* had 23 chromosomes in six roots, one of them was variable: it had also had a few plates with 24 as well as one with 25 chromosomes (Fig. 13); one root had 22, one 22 though also plates with 21 and 24 chromosomes.

An interesting plant was *6—14*. All roots investigated had a high chromosome number. At least two of the roots had 26 chromosomes in all clear plates, while in two other roots plates with 26 and with 25 chromosomes were observed. Two roots had 25 chromosomes while in two roots 25 and 24 chromosomes were seen. In several roots a small fragment was present. Fig. 14 shows 26 + 1 fr., Fig. 15 26 + 2 fr. Thus two fragments may occur, though only rarely. The fragment could not be detected in all plates of a root possessing it. Its small size probably



Figs. 11—19. — *11*: 24 chromosomes. — *12*: 21 + 2 fr. — *13*: 25 chromosomes. — *14*: 26 + fr. — *15*: 26 + 2 fr. — *16*: 23 + fr. — *17*: 21 chromosomes. — *18*: lack of synchronization. — *19*: acentric fragment.

leads to a frequent elimination. One could be inclined to think that the fertilized egg-cell had in this case had more than 23 chromosomes, perhaps 25. This might well be the case. Disturbances perhaps also occur in the mitosis of the embryo-sac, resulting in egg-cells with an unusual high number of chromosomes (perhaps 18). However, *6—14* was the only plant that had more than 23 chromosomes in all investigated roots, thus affording the only evidence of functional gametes with more than the expected chromosome number. Still, the evidence is uncertain, because the rise of the chromosome number may have occurred in the embryo.

About half of the investigated plants (11) seem to have come from fertilized egg-cells having 22 chromosomes. None of them was quite

constant. The most common variation was that a plant had plates or whole roots with 23 chromosomes. Some plants had a few plates or roots with 21 chromosomes. 24 chromosomes were rarely seen in a few plants, for instance, in a root with many 22-chromosome plates one with 24 was observed. A tendency to high chromosome number was shown by *6—16*. Four roots had 22 chromosomes, one had 22 and 23, two had 23 chromosomes.

Fragment chromosomes have already been described from *6—14*. Some other cases were found. Fig. 16 is from *6—15* and shows a plate with a small fragment that was seen in a few plates of a root with 23 chromosomes. Sometimes an arm of a chromosome simulated a fragment (Fig. 17). *6—13* had in one root plates with a very small fragment similar to a satellite.

In this cross four plants had more than 22 chromosomes, four or five had fewer than 22 chromosomes, while eleven or twelve plants had this number. This cross has thus given a different result from the cross described just before. The difference must be due to the fact that the supernumeraries showed a different pairing in the mother plants.

Variation. — The chromosome variation observed and now described was a numerical variation of ordinary chromosomes and the appearance of fragment chromosomes. As the material is unsuited for a study of the morphology of the chromosomes, it is an open question whether structural changes less easy to observe occur, such as small translocations or reciprocal translocations leading to the forming of new chromosomes. There is a certain amount of variation that is of less interest, as it does not consist of permanent changes. RESENDE has shown that satellite chromosomes are subject to some variation. Many of the changes he has described also occur in this material. The size of the satellite may be different in the two »homologous» satellite chromosomes, but still more variable in size is the satellite of the third chromosome set; it is sometimes rather large. The threads carrying the satellites may be long, and are sometimes bent, and when the thread had lost the stain the satellite could erroneously be interpreted as a fragment. Several roots were found that had long satellite threads or a comparatively large satellite in most or many plates. In rare cases the satellite of the third chromosome set was not spherical but cylindrical; a constriction may give it the appearance of a tandem satellite. In one or two roots the satellite chromosomes were unusually long, the two »homologues» belonging to the largest instead of the smallest chromo-

somes of the plate. Here an abnormally weak spiralization had occurred, though seemingly only of the satellite chromosomes.

There may, though rather rarely, be seen a weakly stained or quite unstained segment in a chromosome (Fig. 11). The segment seems to be more easily stretched than the chromosome segments that have a normal charge of nucleic acid. The smaller of the latter segments may be mistaken for a fragment (compare Fig. 17).

The variation in chromosome number in these hybrids is very frequent but of a small amplitude. Polyploidy is rare. Only one plate with the doubled (hexaploid) chromosome number 42 was observed. A second high number also observed once was 34. As a rule, the deviating plates had one chromosome more or less than the majority. A number of plants seemed to have a constant chromosome number, no variation being observed. In other plants a few (one or two) deviating plates were found. In most cases the variation was stronger, being intraradicular or interradicular or both. In some cases the variation was so strong that it was impossible to determine the true chromosome number of the plant, that is, the number of the fertilized egg-cell. However, the variability could not obscure the fact that the result of the three crosses was different, as might have been expected from the different number or pairing of the accessory chromosomes of the mother plants.

Most constant plants had 21 chromosomes. In all, 57 plants were investigated and 15 of them were considered 21-chromosomal. In nine plants no variation was found and only one of them had more than 21 chromosomes. But not all 21-chromosome plants were constant; some of them had a few plates with 22 chromosomes and probably there were also 21-chromosome plants with roots having 22 chromosomes. I found a root with 20 chromosomes, and in intraradicular variations also came across one or two plates with this number. It is nevertheless clear that a lower number than 21 chromosomes only exceptionally occurs. One cannot avoid the conclusion that, though many plants lacking accessory chromosomes did not show any chromosome variation, some of them had some plates or even roots with one chromosome more or (rarely) less than the normal number.

But no doubt the chromosome instability is more pronounced when accessory chromosomes are present. In probably 42 plants the true number was more than 21, but in only one of them was no variation found. A root with predominantly 22 chromosomes might show plates with 21 or with 23 chromosomes. Much more rare were 24-chromosome plates. In one root the only deviating plate had this chromosome num-

ber, and perhaps the disturbance had involved two chromosomes in the same mitosis. Probably more frequently the number 24 comes from the division of a nucleus having 23 chromosomes. The interradicular and the intraradicular variation was very similar; roots with the deviating numbers 21 and 23 often occur. On the other hand, it cannot be doubted that different plants show a different variation; some show only few deviating plates, while other plants are very variable. An exceptionally strong variation was shown by a root with 21—24 chromosomes.

In plants having 23 as predominant chromosome number there are much more often found plates with 24 chromosomes. Such plants may also have roots with 24 chromosomes, though this is more rare. Plates with 25 chromosomes are very rare. Plates or roots with 22 chromosomes may occur, but plates having only 21 chromosomes were only observed in the most variable plant. Only one 23-chromosome plant was very variable, while two had a small and three an intermediate variation. Thus, one does not get the impression that plants having two supernumeraries are more variable than plants with one supernumerary, but the material is not sufficiently large to allow of a definite decision on this point. Plant *6—14* with more than 23 chromosomes in all roots was rather variable, but the variation was mostly interradicular.

Deviating plates may be observed in all parts of the root. It must, however, be stressed that in the central cylinder (plerome) the plates are indistinct and that in the epidermis no plates could be studied, the cells being filled with a fatty substance. This perhaps explains why no periclinal chimæra was observed. Sometimes it was possible to ascertain that a group of cells was aberrant, and a root that was a sectorial chimæra was found, the two halves of the root having different chromosome numbers. In the latter case the root initials had probably had different chromosome numbers (the number of the epidermis cells could, as usual, not be determined).

A second conspicuous kind of variation was the occurrence of fragment chromosomes; they were found in at least eight plants. As a rule, only one of the investigated roots had a fragment chromosome, plant *6—14* being an exception. The size of the fragment chromosome was different. In the plants *6—14* and *8—2* they were very small; in other plants they were larger. In certain cases a fragment chromosome seems to be present in all cells of the root, and plates showing two fragment chromosomes could also be found. In other cases the fragment chromosome was lacking in a minority of the plates, and then it probably had

been lost in some mitosis. However, when a fragment chromosome is present only in a few plates, it must be assumed that it has been formed within the root. One root was a sectorial chimæra having a fragment chromosome only in a large sector occupying about half of the circumference of the root. On the whole, there are many similarities between the occurrence of fragment chromosomes and the occurrence of the numerical variation, which probably indicates that in a certain sense these two forms of variation have a similar cause.

There were some less clear cases of fragment chromosome. One or two roots had a chromosome that was somewhat smaller than the smallest of the ordinary chromosomes. This was perhaps a changed chromosome and might be considered a very large fragment chromosome. In some roots, on the other hand, there was found what seemed to be extremely small fragment chromosomes. However, they were never observed in a large number of plates of the root in question, and the difficulty or impossibility of distinguishing such small fragments from satellites in this material has already been pointed out. There is also the risk that acentric fragments may be interpreted as fragment chromosomes.

Mitotic irregularities. — The study of the chromosome plates showed that irregularities may occur. One or very rarely two chromosomes may sometimes have a faulty orientation, lying in the longitudinal direction of the spindle. It could of course not be ascertained that this was an accessory chromosome, but it was only observed in plates having such chromosomes. Sometimes one or two chromosomes were seen at different foci; such had not reached the plate although the metaphase was rather advanced. Lack of synchronization in the separation of chromatids was not infrequent (Fig. 10). Fig. 18 shows another case: six or seven chromosomes are divided. In certain roots the plates showed a tendency to have chromosomes of different thickness. This probably depended on faulty technique (not on a disturbed nucleic acid metabolism). Chromosome loss was shown by the occurrence of root cells with a chromosome or perhaps part of a chromosome outside the nucleus.

Roots cut lengthwise were more favourable for a study of mitotic disturbances. Roots of 12 plants were studied; four of these plants had 21 chromosomes. Some disturbances were of a more general nature and probably indicated a physiological unbalance. Such was the persistence of the nucleolus or large parts of the nucleolus at early metaphase; the persistent nucleolus rest was often oblong and in most cases still in

Figs. 20—32. — *20*: chromosome configuration lagging between the anaphase groups. — *21*—*22*: bridge configuration. — *23*—*25*: broken bridge configurations. — *26*: bridge and acentric fragment. — *27*: eliminated chromosome. — *28*—*30*: lagging. — *31*: early reproduction in lagging chromosome? — *32*: fragmentation of a chromosome.

contact with chromosomes. Non-congression, one or more chromosomes lying between the metaphase plate and the pole, is also common. These observations are less important and could also be made in plants having 21 chromosomes.

Bridges and chromosome elimination were only observed in roots of plants with more than 21 chromosomes. They occurred in seven of the plants, in one only elimination being observed. In several cases it could be ascertained that the chromosome number in a root showing disturbances of this kind was oscillating. As a rule only few bridges were observed even if the root had a large number of divisions. However,

one region of a root of plant *8—18* had many cells with a bridge, and the behaviour of the bridge could be studied more closely. Here adjacent cells in the same row may have a bridge, indicating repeated formation. Some plates showed that the roots of *8—18* cut lengthwise had cells with 21 and with 22 chromosomes.

In *8—18*, as in the other plants, the bridge configuration had a characteristic appearance. At each end there seemed to be two centromeres, but the middle part of the bridge seemed to be undivided, though sometimes a doubleness could be discerned. The bridge was thus formed of two chromosomes. Fig. 20 shows an early anaphase, the chromosome configuration is left between the separating groups; the undivided middle part is still short. Fig. 21 shows the configuration uniting the telophase nuclei. Fig. 22 shows the daughter nuclei just after the wall has been formed. At wall formation the nuclei approach one another; the bridge is very short, it seems to possess great elasticity. The bridge then breaks and the nuclei move apart. The centromere parts of the configuration may remain outside the nucleus, adhering to the membrane (Fig. 23). Fig. 24 shows a different behaviour of the ends of the bridge; no doubt loss of chromosomes may follow the bridge formation. Fragmentation of chromosomes may also be the result of bridge formation. Fig. 25 shows that the middle part of the bridge is left between the telophase nuclei. This was, however, very rarely observed. In *8—18* only one bridge was observed in the cell. In another plant two bridges once occurred, but this plant had more chromosomes, the root in question having plates with 23 and probably also with 24 chromosomes.

The bridge configuration thus has a characteristic appearance. There is only one configuration in the cell; it occurs in roots with accessory chromosomes. It does not seem possible to interpret the configuration as the accessory chromosome, sometimes behaving irregularly at mitosis, though such an interpretation is tempting. The anaphase bridge resembles certain criss-cross bridges which may be formed after an asymmetrical interchange between two chromosomes; such an interchange also results in acentric fragments (see LEA, 1946), but the *Godetia* bridges are, as a rule, not accompanied by fragments. Hence they have not been formed through breakage and reunion. Therefore they are better compared with the very similar bridges described and figured by GUSTAFSSON (1936). They were in seedling roots from X-rayed seeds of *Hordeum distichum*. The four chromatids at the end of two chromosomes are fused and this fusion is so firm that they are unable to separate. Concerning the cause of this difficulty of separation,

GUSTAFSSON writes »that at the same time as the physiological change in the end of the chromosomes which brings about the fusion, the reproduction has failed, or the four chromomeres located at the point of fusion were so firmly fused that they cannot become detached from each other later on». The first explanation is considered most likely. In *G. nutans* × *Whitneyi* very probably the fusion is always between the same chromosome ends, one of them belonging to the accessory chromosome.

Acentric fragments are rare. Fig. 19 shows such a fragment (the plate was divided by the microtome knife), the spherical body lying between two chromosomes in the plate is probably a satellite. Sometimes a larger fragment seemed to be inserted laterally to or to lie alongside the bridge configuration (Fig. 26). The acentric fragments show that breakage may rarely occur in the resting nucleus.

In rare cases chromosomes lag at the equator at rather late anaphase. Such a case is shown in Fig. 28; the centromere of the chromosome between the anaphase groups had perhaps a faulty orientation in the spindle. In Fig. 30 chromatids are lagging. Fig. 29 shows two lagging chromosomes, the one to the right seems to be a chromatid. Lagging may result in elimination. Microcytes have been observed, but in other cells the eliminated chromosome had not formed a nucleus. Perhaps in the latter cases deficient chromosomes lacking centromere were eliminated as a result of bridge formation.

Sometimes a chromosome lagging between the anaphase groups had a rather peculiar appearance (Figs. 31 and 32). It is difficult to interpret these changes in the chromosome, presumably the accessory chromosome. Fig. 32 seems to show a sort of fragmentation of the chromosome. The case in Fig. 31 is perhaps a non-disjunction of the configuration or an early reproduction in the chromosome.

CONCLUSIONS.

Earlier investigation of *Godetia Whitneyi* or *G. nutans* has not shown any tendency to intra-individual chromosome variation. Such has only been observed in the hybrids now studied. The hybridization accordingly seems to be of importance. I have earlier studied the cytology of many *nutans* × *Whitneyi* hybrids, though in most cases only meiosis, but have observed no chromosome variation. The plants studied earlier were as a rule from crosses with *nutans* plants lacking accessory chromosomes. Chromosome variations were now observed in almost all

hybrid plants with a supernumerary chromosome, while most plants lacking supernumeraries seemed to be constant. On the other hand, variation must be absent or very rare in plants of *G. nutans* with a supernumerary, only one root with aberrant chromosome number being found in a parallel investigation. Hybridization and accessory chromosomes are two factors influencing mitosis in this case, their combined action calling forth the irregularities described.

The hybrids are allotriploid but two of the chromosome sets are very similar. At meiosis they always form seven homomorphic bivalents. It is true that in the roots morphological analysis of the plate is impossible; clearly, however, two of the satellite chromosomes are identically alike. The third chromosome set is always unpaired in the hybrid meiosis and its satellite chromosome has a different size. The hybrid may cytologically be considered as a *G. Whitneyi* having a foreign set of chromosomes extra. Probably the cells in such a hybrid are less sensitive to chromosome aberrations than the cells of an ordinary diploid form or a hybrid with all chromosome sets dissimilar. Changes in or losses of chromosomes belonging to the third set or of the supernumerary chromosome would not be expected to be lethal. It is, however, a fact that plates or roots with less than 21 chromosomes are very rare, but this may depend on lack of or rarity of disturbances in plants with 21 chromosomes.

In these hybrids it was observed that root cells or whole roots had a deviating chromosome number, as a rule one more or less than the normal. This numerical variation could be explained by non-disjunction or loss or may have other causes. The presence of fragment chromosomes shows other changes. The anaphase bridges were perhaps a result of a peculiar kind of stickiness; it must be stressed that as a rule a cell had only one bridge having a rather peculiar appearance. The bridges probably play a rôle in the causation of the chromosome variation. Acentric fragments are rare and a ring chromosome was never observed. Thus, apart from the bridges there is little evidence of chromosome change during the resting stage.

A survey and study of spontaneous chromosome change were made by DARLINGTON and UPCOTT (1941). They studied the first mitosis in pollen grains, and especially *Tulipa fragrans* (a $3x$ form) showed many structural changes. These had arisen in the preceding meiosis or in the resting nucleus. The authors cite some unpublished observations made by different researchers of bridges and fragments in root tips (*Pæonia peregrina*, *P. corallina*, *Pisum sativum*, and *Allium cepa*). Ring chromo-

somes and bridges accompanied by fragments were observed by NICHOLS (1941) in *Allium cepa.* They did not occur in roots from bulbs, only in roots from seeds. The aberrations could be rather frequent, the percentage being different in different forms of *A. cepa* and higher in roots from old seeds. They were more frequent than in roots of *Tradescantia,* investigated by GILES (1940 and unpublished). In *Nothoscordum fragans* there are no disturbed mitotic divisions in roots from bulbs; in roots from young seedlings, however, ring chromosomes, dicentric chromatids and acentric fragments may occur (D'AMATO, 1948). NICHOLS and D'AMATO stress the importance of dehydration, the relatively hydrated cells from bulbs showing no breaks. Seedlings from very old seeds of *Crepis, Datura* and other objects are well-known cases of frequent spontaneous changes. It seems in all these cases always to be breakages of the chromosomes, often followed by reunions of different kind taking place in resting stages. Reciprocal translocations also occur (in *Crepis*). Structural change may also take place late in the ontogeny in premeiotic divisions, and the result is observed in some pollen mother cells of the anther. A survey of spontaneous aberrations of chromosome size and form in *Zea* has been made by MCCLINTOCK (1941). The behaviour of ring chromosomes, of broken ends and of a chromosome with a strictly terminal centromere is interesting.

Intra-individual variations of the number of chromosomes have of course been recorded many times. So have polyploid changes; they occur in most cases (certain haploids being an exception) in a low frequency, some have been listed by DARLINGTON (1937). They are rare in this material. The peculiar feature of the numerical variation in the *Godetia* hybrid, a low amplitude but a high frequency, seems to be rather characteristic of this material. Frequent but large variations in number have been found in colchicine-induced tetraploid *Ribes nigrum* (VAARAMA, 1949). In root tip meristeme and in young ovules 4—32 chromosomes could be observed. As the normal number is 32, there is a loss of chromosomes, as a rule through the forming of more than one spindle in the cell. In roots of timothy plants with a very high chromosome number LEVAN (1949, p. 47, and unpublished) has observed disturbed mitosis, resulting in sectors with deviating chromosome numbers. Tripolar spindles could cause gross changes in number. The disturbances were observed in $12x$ and $13x$ plants but not in $11x$ plants or plants with lower numbers. Disturbed mitosis has also been observed in roots of *Gladiolus* (JONES and BAMFORD, 1941). In some plants from crosses triploid $\times$ diploid and triploid $\times$ tetraploid certain areas

had a rather different chromosome number from surrounding tissue (76—88), roots that seemed to be sectorial chimæras were also found. Observed disturbances were bridges, premature division of chromosomes, and persistent nucleolus. The authors cite cases of mitotic disturbance in *Arachis hypogœa* (BABA) and a clone of *Hemerocallis fulva* (STOUT and SUSA).

A rather strong numerical variation has in some cases been discovered by the study of meiosis in pollen mother cells. LOVE (1938) studied meiosis in 336 plants derived from a pentaploid wheat hybrid. In 16 plants a number of pollen mother cells had less than the $2n$ number of chromosomes of the plant, as few as 20 instead of 42 chromosomes could be observed. Hybridity or aneuploidy could be held as responsible for this variation, but LOVE stresses the fact that the remaining 320 hybrid plants, 184 of which were unbalanced aneuploid types, showed complete regularity. Atypical mitosis that must have occurred rather late in the ontogeny is considered the cause of the changed chromosome numbers. LOVE has also recorded two haploid pollen mother cells in a 7th generation derivative of a *Triticum vulgare* cross, the rest of the pollen mother cells having 42 chromosomes. HOLLINGSHEAD (1932) observed rare cases of hypoploid pollen mother cells in hybrids between *vulgare* wheats. KATTERMANN (1933) found hypoploid pollen mother cells in wheat-rye hybrids. He attributed these and certain other irregularities to cytomixis, and thus this chromosome variation should have quite a different cause from the others described. Small numerical variations seem to be reported more rarely; they are of course less easily detected.

PLOTNIKOWA (1932) describes mitotic disturbances in root tips of wheat-rye hybrids. It was, however, in plants from the 5th or 6th generations quite similar to wheat or with some characters of rye. Lagging chromosomes, bridges and eliminated chromosomes were observed. Only once was there a »tetraploid» plate in the 600 roots investigated. Some plants showed rather many disturbed divisions, in other plants only regular divisions were seen. The authoress mentions that PROSINA has observed »sehr ähnliche Erscheinungen in *Helianthus*». It is not recorded whether these disturbances result in changes of chromosome number. This is perhaps the case; such changes may cause small and frequent deviations. In rare cases small deviations are detected as whole shoots like the $2n$—1 shoots in plants of *Zea*. Several cases are known where one or a few pollen mother cells had one chromosome more or less than the rest. During the investigation of meiosis in sixteen *G. Whit-*

neyi plants having a *deflexa* chromosome extra I found that no less than three had anthers or whole buds lacking this chromosome (HÅKANSSON, 1941). The foreign chromosome, thus, is easily lost in premeiotic division. In inbred rye MÜNTZING and AKDIK (1948) observed some pollen mother cells containing an additional body that appeared like »a small but otherwise typical bivalent». More cases will not be cited.

After crosses between different species of animals it has sometimes been observed that the first divisions of the hybrid nucleus during the cleavage of the egg or in the young embryo are much disturbed and lead to the forming of cells with a low chromosome number (SVÄRDSON, 1945). In many cases the chromosomes contributed by the male parent are eliminated (HERTWIG, 1936). No parallelism to this has been found in the vegetable kingdom. Nor has it been shown that species hybrids of plants show mitotic irregularities particularly often. However, it is probably a general opinion that a chromosome condition unbalanced through hybridization may predispose to mitotic disturbances, particularly perhaps in cases where the foreign chromosome sets are in different numbers, as in the allotriploid now investigated.

There are mitotic irregularities that are a result of the peculiar structure of a certain chromosome as, for instance, a ring chromosome, or a strictly telomitic chromosome (see MCCLINTOCK, 1941); other irregularities may occur »under genetic control». The irregularities here described were called forth through the combined action of hybridization and accessory chromosomes. An aberrant behaviour of certain accessory chromosomes in the first mitosis of the pollen grain leading to non-disjunction is known, and also an elimination of accessories from certain somatic divisions (see MÜNTZING, 1949). Genetic factors, but also a special structure, seem to be the reasons for the aberrant behaviour of accessories. It is a great disadvantage that the supernumerary chromosomes of *Godetia nutans* (and of *G. viminea*; HÅKANSSON, 1949) cannot be distinguished from the A chromosomes. These chromosomes show only some of the properties of typical accessories. It is, however, very probable that the accessory chromosome is involved in the bridge configuration and in this way influences the mitosis more directly through a peculiar structure. In the pure species no aberrant behaviour was observed, the cellular *milieu* or the genotype perhaps being more favourable to this chromosome.

SUMMARY.

Root tips of F_1 hybrids between *Godetia nutans* plants having one or two supernumerary (accessory) chromosomes and *G. Whitneyi* were investigated. They ought to have 21 A chromosomes and perhaps also accessory chromosomes.

Most plants had a varying number of chromosomes, different roots may have different numbers, and the cells within the same root may show variation. The amplitude of variation is as a rule low, ± 1. The frequency of variation was different in different plants and in different roots. Plants with 21 chromosomes were constant or nearly constant with perhaps a few exceptions.

Fragment chromosomes occurred in a number of roots. Their size was different in different plants. Bridges and chromosome elimination were found in roots having supernumerary chromosomes and numerical variation, they were absent in plants lacking such chromosomes.

The result of the crosses is such as could be expected from the number and pairing of the supernumeraries of the mother plants. One plant, however, had many chromosomes, 24—26.

In *G. nutans* plants with accessory chromosomes no irregularities occurred.

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THE FEULGEN TEST APPLIED TO CENTROMERIC CHROMOMERES

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A definite structure in the centromere of the pachytene chromosomes of rye was observed by the present writer (LIMA-DE-FARIA, 1949 a). This structure is characterized by the existence of fibrillæ and chromomeres quite similar in appearance to those existing in the arms of the chromosome and by the disposition of these fibrillæ and chromomeres in the form of a reversed repeat (LIMA-DE-FARIA, 1949 b).

This structure furnished the material basis that permitted a better understanding of the behaviour of fragment chromosomes of maize arising after breakage through the centromere. It also gave the material foundation for an interpretation of the genetics, origin and evolution of kinetochores (LIMA-DE-FARIA, 1949 a and b).

The fact that no *essential* difference in a morphological respect can be detected between the structure of the centromere of the pachytene chromosomes of rye and the structure of the arms of the same chromosomes led to the main assumption that the centromere is a specialized region of the chromosome body and not a »specific organ» localized as a foreign enclave in the chromosome (NAVASHIN, 1932; DARLINGTON, 1946, and others).

This assumption does not necessarily imply that the centromere may have the same submicroscopic structure or chemical constitution as the euchromatin of the chromosome arms. The centromere must have to a certain extent a different chemical constitution owing to its differential behaviour in relation to the rest of the chromosome; but during what stage (or stages) of the chromosome cycle and at what investigation level this difference is apparent can only be shown by further research. To gain an insight into this problem the present investigation was carried out.

I. MATERIAL.

In rye this structure of the centromere is very distinct when it is apparent, but can only be seen in a few preparations showing a partic-

ularly good staining. For this reason a search was made for another material in which chromomeres at the centromere could be found more easily.

BELLING (1928) reported the existence of a »conspicuous spherule» at the centromere of the chromosomes of *Agapanthus umbellatus* observed at pachytene. In this species the centromere can be very easily identified at pachytene owing to a very marked thickening of the chromosomes on both sides of the centromere, the rest of the arms remaining thin. This fact permitted BELLING to identify in a single cell the fifteen centromeric regions corresponding to the 15 bivalent chromosomes of this plant.

DARLINGTON (1933) reinvestigated this case (using the same preparations, lent him by BELLING). He described the »spherule» as the »attachment chromomere».

As this material seemed to be easier to investigate than rye, I decided to study the chromosomes of *Agapanthus umbellatus* at pachytene, with a view to apply to them the FEULGEN test.

The plants used were two specimens growing in the Botanical Garden of the University of Lund.

II. METHODS AND OBSERVATIONS.

1. THE STAINING OF THE CHROMOSOMES WITH IRON-ACETO-CARMINE.

Before the FEULGEN reaction was applied, preparations stained with iron-aceto-carmine were made to reinvestigate the structure of the centromere in this species.

The details of the technique employed were the same as those in that used for the study of the chromosomes of rye at pachytene (LIMA-DE-FARIA, 1948), with the exception that, in this case, no previous fixation in acetic-alcohol was made. The buds were removed from the inflorescence and an anther was immediately placed in a drop of iron-aceto-carmine, from which the P.M.C.'s were extracted with the help of two needles. After heat and pressure had been applied the preparations were made permanent.

The pachytene chromosomes show a perfect contrast to the cytoplasm, and the chromomeres are very distinct. The chromosomes are thin near their extremities and distinctly thick close to the centromere. This differential thickening of the chromosome permits a sure identi-

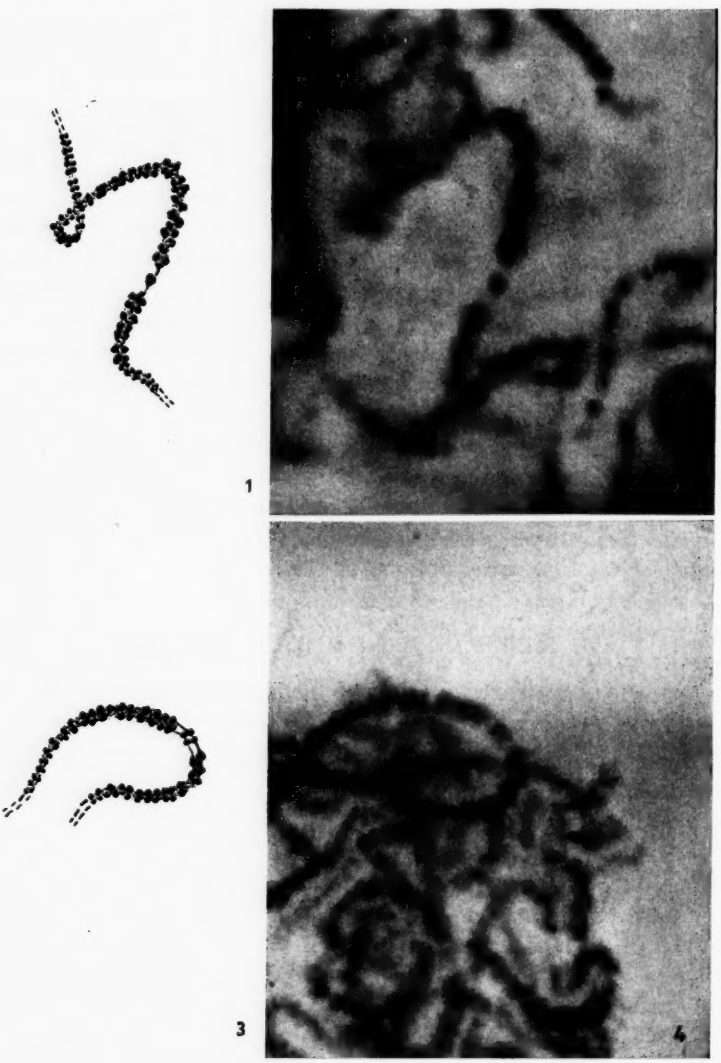


Fig. 1, camera lucida drawing of part of a chromosome pair of *Agapanthus umbellatus* at pachytene, showing a chromomere at the centromere. — × 2500. — Fig. 2, photomicrograph of the pair of chromosomes drawn in Fig. 1. — × 3300. — Fig. 3, camera lucida drawing of part of another chromosome pair showing two pairs of chromomeres at the centromere. — × 2500. — Fig. 4, photomicrograph of the pair of chromosomes drawn in Fig. 3. — × 3250 (*cf.* BELLING, 1928 and DARLINGTON, 1933). — Figs. 1—4 made from preparations stained with iron-aceto-carmine.

fication of the position of the centromere in the chromosome body (Figs. 1 and 3; *cf.* BELLING, 1928, Fig. 3).

No knobs can be observed in the chromosomes.

In one preparation (No. 506) the centromere can be seen to be composed of a fibrilla, a chromomere (usually egg-shaped) which seems to be compound transversally and longitudinally, and another fibrilla (Figs. 1 and 2; *cf.* DARLINGTON, 1933).

In another preparation (No. 517) made from another anther of the same plant, the structure of the centromere is much more distinct and the transversal and longitudinal doubleness of the centromeric chromomeres is clearly perceptible. Considering the two paired chromosomes, the following sequence can be observed (starting from one extremity of the centromere): Two slightly stained fibrillæ, a pair of chromomeres, another pair of chromomeres, and again two slightly stained fibrillæ (Figs. 3 and 4). The centromeric chromomeres are double not only transversally, corresponding to the two paired chromosomes, but also show a longitudinal doubleness corresponding to a longitudinal differentiation inside the same chromosome. This structure was observed with perfect distinctness in two cells and less clearly in six other cells.

The difference in the structure of the centromere found in the two preparations may be explained by assuming a different degree of contraction of the pachytene chromosomes in the two cases. BELLING (1928) suggested that, in *Lilium*, the chromomeres of the arms may approximate, decreasing in this way the total length of the chromosome. He also thought that the thickening of the chromosomes observed in the neighbourhood of the centromere of *Agapanthus* was partly due to such an approximation of the chromomeres.

The structure of the centromere of the pachytene chromosomes of rye could be divided into three zones, *viz.* (1) A first zone, the exterior zone, in which the two fibrillæ are almost unstained. This exterior zone forms on both sides of the centromere the connection with the chromomeres of the arms. (2) A second zone, the chromomeric zone, composed of two pairs of centromeric chromomeres. These two pairs of chromomeres are disposed in longitudinal sequence and each pair is attached to the two almost unstained fibrillæ that compose the exterior zone. (3) A third zone, the interior zone, comprises the space between the two pairs of centromeric chromomeres; two slightly stained fibrillæ can be observed in it. This zone is characterized by a deeper staining than the first zone (LIMA-DE-FARIA, 1949 a).

By comparison with the above description given for the centromere of *Agapanthus* it is possible to see that the first and second zones observed in rye find a perfect correspondence in the centromere of *Agapanthus*, but that the third zone could not be observed in these preparations, the two pairs of chromomeres being situated close together. However, DARLINGTON (1933) has a figure of a cell (Fig. 9 B) in which a single chromosome pair of this cell shows the two pairs of centromeric chromomeres longitudinally separated by a fibrilla. There is no reference in his paper to this third zone, but the figure does not leave any doubt of its homology with the corresponding zone found in rye. This fact suggests that when the chromosomes are still less contracted the two pairs of chromomeres may show their fibrillar interconnection. DARLINGTON has drawn a single fibrilla in this third zone. In the rye chromosomes this zone was more deeply stained than the first zone, which made visual separation of the two fibrillæ more difficult. This fact may account for DARLINGTON's drawing of one single fibrilla, or most probably, they were so close together that they could not be distinguished. Hence, it seems that the same zone in *Secale* and *Agapanthus* displays the same morphologic features.

When making reference to the staining intensity of the centromeric chromomeres, BELLING (1928) compares them with the adjacent chromomeres of the thicker zone of the chromosome situated on both sides of the centromere, and states that the »spherule» stained brown (with iron-brazilin) while the chromomeres on both sides stained black. This was not the most accurate way of comparing the colour of the centromeric chromomeres with the other chromomeres of the arms, because on both sides of the centromere the chromosome is particularly thickened. DARLINGTON (1933), more accurately, compares the centromeric chromomeres with the rest of the chromomeres of the chromosome and states that they are usually less deeply stained than the other chromomeres. In the preparations of the present investigation the centromeric chromomeres are less deeply stained than the chromomeres situated in the neighbourhood of the centromere but more intensely than the chromomeres of the middle and distal parts of the arms.

The difference between the two observations may depend upon the two different dyes used. BELLING's (and DARLINGTON's) preparations were stained with iron-brazilin, those of the present investigation with iron-aceto-carmine.

When preparations of pachytene chromosomes were made, not by directly teasing out the anthers in iron-aceto-carmine, but by previous

fixation of the buds in acetic-alcohol (1 : 4, for 7—9 hours; transference to 95 % alcohol, over-night; storage for a few days in 70 % alcohol) and subsequent staining in iron-aceto-carmine, the structure of the centromere was not apparent. The centromere appears as a faintly stained region marked by some darker dots that suggest chromomeres, though no definite statement can be made about its structure. This appearance is very similar to the one observed in rye in most preparations treated in the same way (previous fixation in acetic-alcohol 1 : 4 for 4 hours; transference to 95 % alcohol, over-night; storage in 70 % alcohol and staining in iron-aceto-carmine).

BELLING's (and DARLINGTON's) material was fixed in a modification of S. NAVASHIN's fixative, which BELLING found satisfactory for demonstrating chromomeres (BELLING, 1930, p. 244). As will be seen below, I used this same fixative when employing the FEULGEN reaction. However, I made a previous fixation in acetic-alcohol before using BELLING's fixative, the result nevertheless being quite satisfactory and the chromomeres being clearly perceptible (see below). Hence, it seems that fixation in acetic-alcohol does not destroy the structure of the centromere but places the centromere in such a physico-chemical state that it generally loses its ability to be stained by most dyes used.

Acetic-alcohol alone is certainly not the best fixative to use when studying the centromere structure, at least in some plants. The homogeneous and structureless appearance of the centromere of pachytene chromosomes of maize may have its origin in the fixative used (acetic-alcohol 1 : 3, for 12—24 hours; MCCLINTOCK, 1929 and 1934, Fig. 1).

2. THE STAINING OF THE CHROMOSOMES BY THE FEULGEN REACTION.

From the preparations stained with iron-aceto-carmine it could be seen that the centromere structure was readily observable in the pachytene chromosomes of *Agapanthus umbellatus*. For that reason this material seemed to be suitable for an application of a microchemical reaction which could reveal part of the chemical constitution of the centromere. The FEULGEN reaction or FEULGEN test was applied.

SCHIFF's reagent (fuchsin-sulphurous acid) can be used in cytological technique in two ways: (1) as a simple stain for chromosomes, or (2) as a specific reagent for desoxyribose nucleic acid when applied under defined conditions.

When SCHIFF's reagent is used as a simple stain for chromosomes several details of the FEULGEN reaction can be omitted or replaced by

others. That is the case in the subsequent modifications of HEITZ's *Nucleal-Quetschmethode*, where the hydrolysis is not only utilized to liberate the aldehyde groups of desoxyribose, but also to bring out the dissolution of the pectic middle lamella, allowing the cells to separate and in this way permitting a better squash of the treated tissue. In this technique the use of the SO_2 water is not strictly necessary, and after being stained in fuchsin-sulphurous acid small pieces of tissue are teased out in 45 % acetic acid (DARLINGTON and LA COUR, 1942). Under these conditions the stain formed on the chromosomes is of use for cytological studies but gives no information concerning the presence or absence of desoxyribose nucleic acid in the chromosomes.

Fuchsin-sulphurous acid is not by itself a specific reagent of desoxyribose nucleic acid, because it can form a dye with substances present in the cell like some polysaccharides (after oxidation; BAUER, 1933), lipids, *etc.* In these substances the colour is developed without previous hydrolysis.

The possibility of applying fuchsin-sulphurous acid as a specific reagent of desoxyribose nucleic acid also lies in the differential behaviour towards weak acid hydrolysis shown by the nucleic acids of the nucleus and those of the cytoplasm (FREY-WYSSLING, 1948). The nucleic acids of the nucleus are very sensitive to weak acid hydrolysis, whereas those of the cytoplasm (ribose nucleic acid) are not. After such a hydrolysis the aldehyde groups of the nucleic acids of the cytoplasm remain masked.

Accordingly, it will be understood that we can only speak of the FEULGEN reaction as a *test* for desoxyribose nucleic acid when an unhydrolysed control is made. If the unhydrolysed control, after immersion in SCHIFF's reagent, shows the formation of a dye at the body in question, we are in presence of a substance which is not desoxyribose nucleic acid and that may be, for instance, a polysaccharide; if on the contrary the unhydrolysed control does not show the formation of any dye and the hydrolysed material develops the colour characteristic of the »regenerated basic fuchsin», it is considered that it reveals the presence of desoxyribose nucleic acid.

This is the way in which the FEULGEN reaction is at present understood as a microchemical test for desoxyribose nucleic acid (SERRA, 1943; STOWELL, 1945; DI STEFANO, 1948).

The technique used in the application of the FEULGEN test to the centromeric chromomeres of *Agapanthus umbellatus* was as follows.

(1) The buds were fixed in 1 : 4 acetic-alcohol for 7—9 hours,

transferred to 95 % alcohol (over-night) and stored in 70 % alcohol for 2—3 days.

(2) Under a wide-field binocular microscope the anthers were cut into two pieces. By gentle pressure made with two needles over the anther-halves the P.M.C.'s were squeezed out in a drop of 45 % aqueous acetic acid.

(3) After all pieces of anther wall had been removed the cover-slip was placed in position and pressure applied over it with a needle curved at its point into a U-shape (LIMA-DE-FARIA, 1948). Neither cover-slip nor slide were filmed. The treatment by steps (1), (2) and (3) was made to flatten the cells before they were immersed in a hardening fixative, as NAVASHIN's fluid.

(4) The slide was gently heated over a spirit flame (once).

(5) Slide and cover-slip were separated by turning the slide face down in a smearing dish containing 10 % aqueous acetic acid.

(6) After the cover-slip had fallen off (3—5 minutes) both cover-slip and slide were transferred to a smearing dish containing BELLING's modification of NAVASHIN's fixative (BELLING, 1930, p. 244), where they were left immersed for three days.

(7) A wash of one hour in distilled water was performed owing to the presence of formalin in the fixative.

(8) The cover-slips to which the P.M.C.'s had stuck were immersed in normal HCl at 60° C. for six minutes.

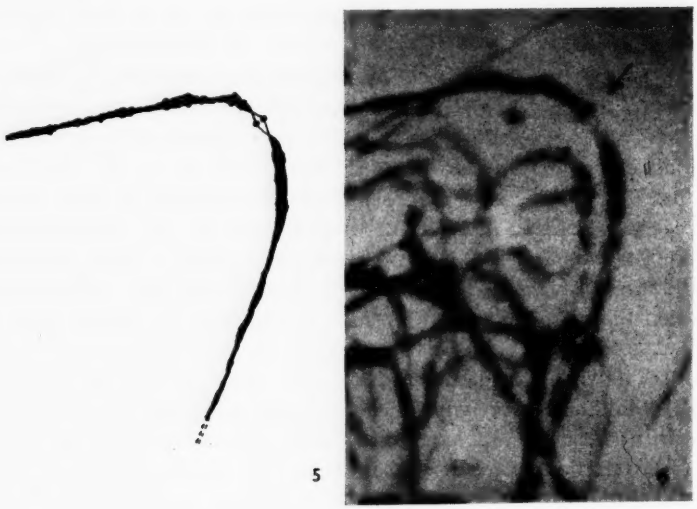
(9) After a brief wash in distilled water (1—2 minutes) the cover-slips were immersed in fuchsin-sulphurous acid for three hours and 30 minutes.

(10) They were then passed through fresh SO_2 water, three changes of ten minutes each.

(11) Finally the cover-slips were rinsed in distilled water, passed through the alcohol series 20 %, 50 %, 70 %, 95 % and left for two to three minutes in absolute alcohol. After that they were mounted in »Euparal», each over a new slide.

Some precautions were taken to ensure a perfect technique, *viz.* (1) After previous fixation in acetic-alcohol and subsequent squash of the pollen mother cells, these were fixed once more in a NAVASHIN's fluid, which BELLING found satisfactory for demonstrating chromomeres. (2) The used sample of fuchsin-sulphurous acid was completely transparent. (3) The SO_2 water was prepared at the moment it was used.

The study of the preparations made by this technique shows the chromosomes stained with the characteristic violet red colour of the



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Figs. 5, 7 and 8, camera lucida drawing of part of three different pairs of pachytene chromosomes showing a pair of chromomeres at the centromere. — × 2500. — Fig. 6, photomicrograph of the pair of chromosomes drawn in Fig. 5, the pair of centromeric chromomeres is indicated by an arrow. — × 3100. — Figs. 5—8 made from preparations stained by the FEULGEN reaction.

dye formed on the chromosomes by SCHIFF's reagent and the cytoplasm completely transparent.

The chromomeres of the arms cannot be distinguished from each other so clearly as in the iron-aceto-carmine preparations, the contour of the chromosome being more homogeneous (Figs. 5—8). However, the centromeric chromomeres, being intercalated among longer fibrillæ than the chromomeres of the arms, are perfectly distinguishable (Figs. 5—8).

As reported above, the centromere region can be detected in the

paired chromosomes with perfect certainty owing to the increase in thickness of the chromosomes on both sides of the centromere shown by the 15 bivalents. If we start from one extremity of the centromere, considering the two paired chromosomes, the following structure can be seen: two slightly stained fibrillæ, a pair of chromomeres, and again two slightly stained fibrillæ (Figs. 5, 6 and 7; in Fig. 8 only one fibrilla can be distinguished on one side of the centromeric chromomeres). The centromeric fibrillæ appear very slightly stained but quite distinct, diverging from each other on the side of the centromeric chromomeres (Figs. 5 and 7). The centromeric chromomeres form a pair, the two elements of which are slightly separated from each other. This structure was observed in six cells of preparation No. 553.

No longitudinal doubleness of the centromeric chromomeres could be observed in the preparations available. If a large number of preparations had been secured, the longitudinal doubleness of the centromeric chromomeres would probably have become apparent, as in the case of the preparations stained with iron-aceto-carmine.

The centromeric chromomeres have a weaker staining intensity than the chromomeres of the arms located in their neighbourhood, but are as deeply stained as the chromomeres of the distal part of the arms.

To test whether the dye formed at the centromeric chromomeres could uncover evidence of the existence of desoxyribose nucleic acid at these chromomeres an unhydrolysed control was made.

This control preparation was made by performing in the same way all steps from (1) to (7), but step (8) was eliminated and the cover-slip to which the P.M.C.'s had stuck was taken from the distilled water and directly immersed in fuchsin-sulphurous acid for 15 minutes. The control material must not remain immersed in fuchsin-sulphurous acid for more than 15—30 minutes, because if a longer time is used the acidity of the SCHIFF's reagent may be, by itself, sufficient to hydrolyse the pollen mother cells (SERRA, *l. c.*). After that, a permanent preparation was made, in the same way as the other preparations, by following steps (10) and (11).

Examination of the control reveals the cells to be completely transparent; only the cell-outline can be distinguished on closing the iris diaphragm. A careful inspection of the cells does not reveal any stained formation, all the cell-content being transparent.

Another source of control was furnished, in the hydrolysed preparations, by the nucleoli. In the iron-aceto-carmine preparations the nucleoli, as is normally the case, take up the dye like the chromosomes,

showing a good contrast to the cytoplasm. In the hydrolysed preparations stained with fuchsin-sulphurous acid, the nucleoli are invisible, as is to be expected in a FEULGEN reaction correctly performed.

As reported above, the outline of the arms of the chromosomes is rather homogeneous in the hydrolysed preparations, which does not permit so clear a separation of the chromomeres as in the preparations stained with iron-aceto-carmine. This fact may be explained in two ways, *viz.* (1) by assuming that a certain degree of coalescence has taken place between the chromomeres of the arms or (2) by supposing that the desoxyribose nucleic acid of the chromomeres of the arms has partially »melted». If this second assumption should be correct, the staining of the centromere (fibrillæ and chromomeres) could be explained on the basis of this phenomenon. As the region in the neighbourhood of the centromere is the thickest of the chromosome, it might be thought that the desoxyribose nucleic acid stained there might run off through the centromere.

Preparations which were hydrolysed during less time than those described above, only one and two minutes, show the chromomeres of the arms better separated from each other but more faintly stained. What has exactly happened in the chromomeres of the arms may be difficult to ascertain, but what is of significance in the present case is to know to what extent a supposed »melting» of the desoxyribose nucleic acid of the arms could account for the staining of the centromere. Such a supposed »melting», if it has taken place in the chromosome arms, cannot account for the staining of the centromere, there being three observations that invalidate such an assumption.

Firstly, if it should be so, the fibrillæ of the centromere would be more deeply stained than the centromeric chromomeres which are situated in the middle of the centromere. This is not the case; the centromeric chromomeres are more deeply stained than the centromeric fibrillæ, and do not appear as a prolongation of the fibrillæ but are sharply distinct from them.

Secondly, if the centromere should have been stained by such a process, the nucleoli would in the same way have got a certain quantity of stain run off from the adjacent part of the chromosome; such a phenomenon could not be observed.

Thirdly, the centromeric chromomeres are as deeply stained as the chromomeres of the distal part of the arms, which affords additional evidence against such an assumption.

As these three observations contradict this supposition, it must be

assumed that the dye appearing at the centromere was formed *in situ* and had not its origin in the arms of the chromosome.

STEDMAN and STEDMAN (1943, 1947) have tried to attribute another significance to the FEULGEN reaction. Their main claim is that the fuchsin-sulphurous acid forms a dye with desoxyribose nucleic acid but that this dye is taken up by the acidic protein »chromosomin», which is said to form the principal component of the chromosomes. According to these authors, it is a fallacy to suppose that the FEULGEN reaction indicates the presence of desoxyribose nucleic acid in the chromosome.

This view received much adverse criticism from many authors, among others: SERRA (*l. c.*), CALLAN (1943), CASPERSSON (1944), STOWELL (*l. c.*), DI STEFANO (*l. c.*), and RIS and MIRSKY (1949). Their arguments are conclusive enough to dispense with any further treatment here of this question.

The FEULGEN reaction possesses, however, several obscure points. For instance, little is known about the nature of the dye formed in the chromosomes by fuchsin-sulphurous acid, the so-called »regenerated basic fuchsin» (BAKER, 1942; CONN, 1946).

In short, it may be stated that, as far as the FEULGEN test is considered a reliable microchemical reaction to put in evidence the presence of desoxyribose nucleic acid in the chromosomes, the centromere of the pachytene chromosomes of *Agapanthus umbellatus* must be considered to possess this substance.

III. DISCUSSION.

The reinvestigation of the structure of the centromere of the pachytene chromosomes of *Agapanthus umbellatus* studied for the first time by BELLING (1928) and afterwards redescribed by DARLINGTON (1933) reveals — in the iron-aceto-carmine preparations — a similar structure to that found in rye. The structure observed in the centromere of the pachytene chromosomes of *Secale cereale* no longer stands isolated as a single example.

All the three zones of the centromere of rye chromosomes can be found in the centromere of the pachytene chromosomes of this plant. The main feature of this centromere structure — the longitudinal doubleness of the centromeric chromomeres — can also be observed in all distinctness in *Agapanthus*.

The structure found in *Secale* furnished the material basis that permitted a better understanding of the behaviour of fragment chromosomes of maize arising after breakage through the centromere (LIMA-

DE-FARIA, 1949 a). ÖSTERGREN's (1947) interpretation of the mechanism of misdivision also found in this structure a particular support (LIMA-DE-FARIA, 1949 a). The recognition of a similar structure in *Agapanthus* gives further strength to these interpretations.

Similarly, as in rye, the centromere of the pachytene chromosomes of *Agapanthus* has a symmetric structure. If we imagine a plane passing through the middle of the centromere, the latter becomes divided into two parts related one to the other as an object to its image in a plane mirror (Fig. 3). As in *Secale* the centromere appears as a reversed repeat (LIMA-DE-FARIA, 1949 b).

The application of the FEULGEN test to the centromere of the pachytene chromosomes of *Agapanthus* reveals that the FEULGEN reaction is positive in the arms as well as in the centromere.

The centromere not only has the same essential type of microscopic structure as the chromosome arms but also possesses the same kind of nucleic acid.

If the nucleic acid is the same throughout all the chromosome, including the centromere, a qualitative difference between the centromere and the euchromatin of the chromosome arms may only be expected to exist in the way in which the nucleic acid and the protein are arranged in the centromere, or in the protein content itself.

The nature of such a difference between the centromere and the chromosome arms is of particular significance for the problem of the existence of genes at the centromere as well as of the origin and evolution of kinetochores (LIMA-DE-FARIA, 1949 b).

At metaphase of mitosis the centromere appears, in general, as a region unprovided with desoxyribose nucleic acid. This fact has been a main contribution to the idea that the centromere is a »specific organ» enclaved in the chromosome body. However, such a phenomenon may have an explanation that fits, at present, the general case.

The genes contribute to the building up of the phenotype by the production of substances which pass into the cytoplasm. This gene production is supposed to take place mostly in the »resting nucleus». At this stage the chromosomes are swollen and the desoxyribose nucleic acid is evenly distributed in the nucleus (RIS and MIRSKY, *l. c.*).

According to FREY-WYSSLING (*l. c.*), nucleic acids are of relatively uniform chemical constitution and, in their molecular morphology, lack the diversity required by genetics. The proteins, with their specific structure, would be the hereditary substance *par excellence* and not the nucleic acids, as firstly thought.

FREY-WYSSLING suggests that the genes play no active part during mitosis, but are passive. Their operative groups must therefore be reactive in the »resting nucleus» to fulfil their task, but they must be screened off during nuclear division, which might be effected by a loose binding of nucleic-acid groups.

Nucleic acids would screen off the genes during mitosis; although, according to FREY-WYSSLING, this idea would not be incompatible with the rôle played by the nucleic acids in the biological synthesis of proteins (CASPERSSON, 1941).

While I am not prepared to agree that during mitosis nucleic acids play only the rôle attributed to them by FREY-WYSSLING, I admit that, due to the large amount of nucleic acid with which the chromosomes are charged at metaphase of mitosis, there may exist at this stage a certain hinderance to the diffusion of the gene products.

If the centromere carries genes (which is far from proved, but for which there is a certain amount of evidence; LIMA-DE-FARIA, 1949 b), these genes, contrary to the genes localized in the arms of the chromosome, would have their most active period not in the »resting nucleus» but during division, where the centromere is the leading part of the chromosome. The existence of a large amount of desoxyribose nucleic acid at the centromere during mitosis would screen off the diffusion of the gene products formed at the centromere, and for this reason the non-accumulation of desoxyribose nucleic acid in this region of the chromosome would have been favoured by natural selection.

SUMMARY.

(1) The structure of the centromere of the pachytene chromosomes of *Agapanthus umbellatus* is reinvestigated. It possesses the same three zones found in the centromere of the pachytene chromosomes of *Secale cereale* and is composed of fibrillæ and chromomeres with a similar disposition.

(2) The application of the FEULGEN test to the pachytene chromosomes of *Agapanthus* reveals the existence of desoxyribose nucleic acid at the centromere. No dye is formed at this region of the chromosome in an unhydrolysed control.

(3) The centromere of the pachytene chromosomes of *Agapanthus umbellatus* not only has the same essential type of microscopic structure as the chromosome arms but also possesses the same kind of nucleic acid.

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GENETICAL STUDIES ON THE N. III TRANSLOCATION IN PISUM

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INTRODUCTION.

IN 1929 I found that an F_2 generation from the cross Bohnenerbse × Automobil contained a large number of plants with pronounced seed abortion. It was later discovered that those plants which had seed abortion also had about one-half of their pollen grains shrivelled (E. NILSSON, 1933). Further, it was demonstrated by HÅKANSSON (1932) that these plants, besides having five bivalents, had a more or less fixed association of four chromosomes, certainly not so regular as in previously known similar cases of sterility in *Pisum* but none the less typical of what is called semi-sterility. HÅKANSSON (*l. c.*) called such configurations *amphibivalents*, a term that translocation research has allowed — unnecessarily, in my opinion — to fall into disuse.

In the work cited HÅKANSSON denotes this case of semi-sterility as *N.III*, a designation which he later (HÅKANSSON, 1934) gives the typographically deviating form *N3*. In order to get a consistent record of the then more closely known cases of semi-sterility in *Pisum* I distinguished (E. NILSSON, 1936) the types in these that were homozygous for the reciprocal translocation with capitals, the normal type (*i. e.* that with the commonest arrangement of chromosome segments) being then called *prime type A* and the translocation homozygote occurring in the case *N.III* being called *prime type E.* Later, EVA SANSOME (1937) introduced another mode of designation and called, for instance, the normal type »structural type 1» (N.III does not occur here). So far as we have referred to the same type in our cited publications, the synonymy is as follows:

	E. NILSSON	EVA SANSOME
The normal type	Prime type A	Structural type 1
HAMMARLUND's K-line	» » B	» » 2
Thibetan pea, interch.	» » C	» » 3
Extra Rapid	» » D	» » 4

MATERIAL AND METHOD.

After having made an investigation on a small scale in order to trace any linkage between the point of interchange and genes in different linkage groups, prime type E was crossed to lines having alleles from this type belonging to loci that in the experiments mentioned appeared to have shown linkage with the point of interchange. The next year, 1948, the F_1 was back-crossed to the respective normal lines, and hence in 1949 the segregations could be counted.

The lines used as crossing material are listed below together with their gene constitution with respect to the pairs of alleles that come in question for this study.

L. 43	Emerald gem	*wa*
L. 124		*le, wlo, p, pl*
L. 135	Kronært	*fa*
L. 141	Prime type E	*B, St, Le, V, N, Fa, Wlo, P, Pl, Wb, K, Wa*
L. 150	from DE WINTON	*b, st, wb, k*
L. 160		*le*
L. 168	Symbol	*le, v*
L. 176		*n*

The gene designations symbolize the following characters:
B reddish-violet flower, *b* pale rose-coloured flower;
St broad stipules, *st* narrow ones;
Le tall-growing, *le* short;
V with strong membrane, *v* almost without membrane in the pod;
N thin pod-wall, *n* thick, fleshy pod-wall;
Fa flowers in the axil, *fa* apical, dense flower cluster;
Wlo waxy leaf, *wlo* upper side of leaf glossy;
P as *V*;
Pl black hilum, *pl* uncoloured hilum;
Wb waxy, *wb* almost glossy;
K normal wings, *k* reduced wings;
Wa as *Wb*.
List of the crosses with the genes studied in these:

I.	L. 141 × L. 150	*B St Wb K*
II.	L. 141 × L. 168	*Le V*
III.	L. 168 × L. 141	» »
IV.	L. 160 × L. 141	*Le*
V.	L. 141 × L. 124	*Le Wlo P Pl*

VI.	L. 176 × L. 141	*N*
VII.	L. 141 × L. 135	*Fa*
VIII.	L. 135 × L. 141	»
IX.	L. 141 × L. 43	*Wa*
X.	L. 43 × L. 141	»

To avoid the use of space-consuming terms every time a crossing procedure has to be mentioned abbreviated designations will hereafter be employed, *e. g.* back-cross (L. 141 × L. 150) × L. 150 will be denoted I *bc* and the reciprocal combination L. 150 × (L. 141 × L. 150) I *bcr*, and so on.

For computation of the degree of crossing-over use is made of the method of maximum likelihood, and the figures found are evaluated according to the χ^2 test. As guides for the statistical analyses recourse has been mainly had to the well-known works of BONNIER and TEDIN (1940), FISHER (1948) and MATHER (1938, 1944).

THE SEGREGATION FERTILE : SEMI-STERILE.

The theoretical segregation in the progeny from semi-sterile plants is 1 fertile: 1 semi-sterile, and the same naturally applies to the back-cross between the F_1 translocation line × normal line and the normal line (translocation line, respectively). When engaged in translocation studies, however, one soon finds that the deviations from the theoretical proportion may often be considerable.

So far as the present material of 19 individual observations is concerned the proportion found agrees fairly well with the theoretical one. Only in one case (IV *bcr*) does there occur a significant deviation, with $\chi^2 = 6{,}4222$ and $P = 0{,}02 - 0{,}01$. It would be of little benefit to take up space here by enumerating all the individual data, and therefore I will content myself by submitting the totals:

650 fert.: 634 ster. Added $\chi^2 = 22{,}3560$
Total $\chi^2 = 0{,}1994$

χ^2 for heterogeneity $= 22{,}1566$

(with 18 degrees of freedom, not significant)

THE B GROUP.

To the linkage groups and chromosomes respectively dealt with here I am henceforth applying the teminology used by LAMPRECHT

(1948). He calls the chromosome that includes loci *B* and *St* »chromosome III» or the »B chromosome».

As table headings over the four groups of individuals use will hereafter be made of the designations a, b, c, d, which then signify

a = dominant fertile
b = dominant semi-sterile
c = recessive fertile
d = recessive semi-sterile.

Unfortunately the number of individuals at my disposal for an appraisal of the linkage between the point of interchange (here abbreviated P.i.) and the genes *B* and *St* studied here is rather small, among other things because the germinability in just this material is poor. However, as back-crosses are concerned here, it should nevertheless suffice for a tolerably accurate judgment of the mutual positions of the three loci.

	a	b	c	d	χ^2 for *B*	χ^2 for independence
I *bc*	1	13	16	2	0,5000	21,1250***
I *bcr*	0	6	10	1	1,4706	13,2353***
		Added			1,9706	34,3603***
	1	19	26	3	1,6531	34,3061***
		Homogeneity			0,3175	0,0542

	a	b	c	d	χ^2 for *St*	χ^2 for independence
I *bc*	4	13	13	2	0,1250	12,5000***
I *bcr*	3	5	7	2	0,0588	2,8824
		Added			0,1838	15,3824***
	7	18	20	4	0,0204	14,8367***
		Homogeneity			0,1634	0,5457

I *bcr* has germinated only 27 % and I *bc* 64 %, but there is nothing to indicate that this great mortality has been selective. That P. i., *B* and *St* are not inherited independently of one another is beyond doubt. The fact that the χ^2 test does not show this for P. i.—*St* in I *bcr* is doubtless assignable to the great influence of random deviations on so small a number of individuals.

The crossing-over percentage between these three loci in this ex-

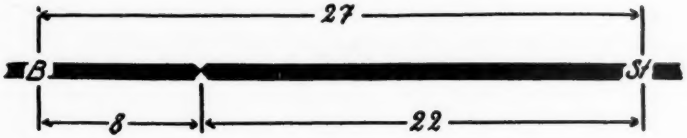

Fig. 1. The linear relation between *B*, P. i. and *St*.

periment is given below, and their position is graphically illustrated in Fig. 1.

P. i.—*B*	8,2
P. i.—*St*	22,4
B—*St*	26,9

In this connection it should be remarked that older F_2 figures show higher crossing-over values between the P. i. and the respective gene than have been found here. The crossing-over percentage has been determined according to LAMM (1948) by means of the method of maximum likelihood (see LAMM, 1948). The L. 119 occurring in the following table but not included in the previously given list of material has rose-coloured flowers (*b*). Other lines referred to in the table have already been mentioned, and the cross L. 141 × L. 150 is identical with cross I.

		C.-o. %	χ^2 for homog.	χ^2 for indep.	n
L. 141 × L. 119 and recipr.	P. i.—*B*	17,1	0,1488	22,1877***	476
L. 141 × L. 150 » »	» »	17,0	0,0119	14,2575***	211
» »	P. i.—*St*	29,1	0,5806	2,5372	»
» »	*B*—*St*	20,5	1,1005	49,6308***	»

THE Le GROUP.

To this group LAMPRECHT (1948) assigns the genes *Le*, *V*, *N*, *Fa* studied here. He intends later on to publish his reasons for bringing together the individually related groups *Le*—*V* and *N*—*Fa*. The linkage *Le*—*V* was first published by BREMER (1926) in an internationally but little known periodical, not cited by RASMUSSON (1927) in his classical treatment of these genes but referred to by WELLENSICK (1929). As regards the detection of linkage between the locus of interchange and *Le*, the following results have been obtained in my experiments:

	a	b	c	d	χ^2 for *Le*	χ^2 for independence
II *bc*	17	46	52	7	0,1311	$44,8852^{***}$
II *bcr*	11	34	37	12	0,1702	$24,5106^{***}$
III *bcr*	0	13	14	7	1,8823	$11,7647^{***}$
		Added			2,1836	$81,1605^{***}$
	28	93	103	26	0,3240	$80,6560^{***}$
		Homogeneity			1,8596	0,5045
V *bc*	13	27	28	16	0,1905	$9,2381^{**}$
V *bcr*	13	25	22	16	0,0000	$4,2684^{*}$
		Added			0,1905	$13,5065^{***}$
	26	52	50	32	0,1000	$12,1000^{***}$
		Homogeneity			0,0905	1,4065
IV *bc*	11	71	60	28	0,0061	$57,0242^{***}$
IV *bcr*	13	7	18	7	0,5556	0,5556
		Added			0,5617	$57,5798^{***}$
	24	78	78	35	0,0422	$49,5429^{***}$
		Homogeneity			0,5195	$8,0369^{**}$

The different crossing groups are kept apart here because, when combined, they show too great a heterogeneity for independence ($\chi^2 = 16,8471$, $P = 0,01$—$0,001$). Even with the above method of summation there is some lack of homogeneity. If the dubious IV *bcr* is excluded and the rest treated as one, χ^2 for homogeneity will equal $12,4060$. The heterogeneity may be derived from the higher degree of crossing-over in V (*vide* below). Still, however the χ^2 analysis is set up, it shows clearly enough the connection between the point of interchange and *Le*. The same also applies, as was expected and as will presently be shown, to the gene *V* that is strongly linked to *Le*.

	a	b	c	d	χ^2 for *V*	χ^2 for independence
II *bc*	10	46	59	7	0,8196	$63,4754^{***}$
II *bcr*	9	39	39	7	0,0426	$40,8936^{***}$
III *bc*	0	15	14	5	0,4706	$16,9412^{***}$
		Added			1,3328	$121,3102^{***}$
	19	100	112	19	0,5760	$121,1400^{***}$
		Homogeneity			0,7568	0,1702

The crossing-over percentages for the complex P. i.—*V*—*Le* are:

P. i.—*V*	II—III	15,2
P. i.—*Le*	IV	20,6
» »	II—III	21,6
» »	V	36,3
Le—*V*	II—III	8,0

There is every reason to believe that the considerably weaker linkage between P. i. and *Le* in cross V is genotypically conditioned, but the point deserves closer investigation.

Unfortunately, I have not had segregation in the same cross of the genes *N* and *Fa* referred by LAMPRECHT (*l. c.*) to the *Le* group. The χ^2 analysis has given the following result:

	a	b	c	d	χ^2 for *N*	χ^2 for independence
VI *bc*	10	12	10	3	2,3142	2,3142
VI *bcr*	21	37	45	30	2,1407	7,6992**
			Added		4,4549	10,0134**
	31	49	55	33	0,3810	9,5238**
			Homogeneity		4,0739	0,4896

	a	b	c	d	*Fa*	independence
VII *bc*	10	15	6	9	2,5000	0,1000
VII *bcr*	29	35	45	29	0,7246	3,5072
VIII *bc*	13	9	17	12	0,9607	0,0196
VIII *bcr*	14	15	14	17	0,0667	0,0667
			Added		4,2520	3,6935
	66	74	82	67	0,2803	1,8304
			Homogeneity		3,9617	1,8631

It is obvious that P. i. and *N* belong to the same chromosome, whereas the χ^2 analysis is not able to disclose any such connection between P. i. and *Fa*. Still, LAMPRECHT (1945) has shown that *N* and *Fa* are linked with 20—25 % crossing-over, and therefore *Fa* must also lie in this chromosome. The crossing-over percentages are

P. i.—*N* 38,1
P. i.—*Fa* ..... 46,0.

If we collocate the facts presented for all the loci dealt with in this chapter, we get the chromosome map submitted in Fig. 2. For P. i.—*Le*

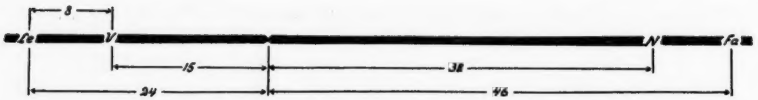

Fig. 2. The linear relation between *Le*, *V*, P. i., *N* and *Fa*.

use has here been made of the crossing-over value given by the sum of the crosses II, III and IV, thus with exclusion of the greatly deviating cross V. The sum total then obtained is 26,8 % crossing-over. Judging from this collocation it may be expected that *N* and *Fa* are rather near each other. In my material, it is true, these genes do not segregate in the same cross, but LAMPRECHT (*l. c.*), as mentioned, has found more than 20 % of the crossing-over between them.

THE Pl GROUP.

This group — LAMPRECHT's chromosome VI or the *Pl*-chromosome (1948) — comprises, among the genes studied here, *Wlo*, *P* and *Pl*. All segregate here in the same cross.

	a	b	c	d	χ^2 for *Wlo*	χ^2 for independence
V *bc*	17	32	24	11	2,3333	9,3333**
V *bcr*	12	30	23	11	0,8421	11,8421***
			Added		3,1754	21,1754***
	29	62	47	22	3,0250	21,0250***
			Homogeneity		0,1504	0,1504

	a	b	c	d	*P*	χ^2 for independence
V *bc*	14	27	27	16	0,0476	6,8571**
V *bcr*	11	27	24	14	0,0000	8,8947**
			Added		0,0476	15,7518***
	25	54	51	30	0,0250	15,6250***
			Homogeneity		0,0226	0,1268

	a	b	c		*Pl*	χ^2 for independence
V *bc*	22	24	19	19	0,7619	0,0476
V *bcr*	14	18	21	23	0,8421	0,0526
			Added		1,6040	0,1002
	36	42	40	42	0,1000	0,1000
			Homogeneity		1,5040	0,0002

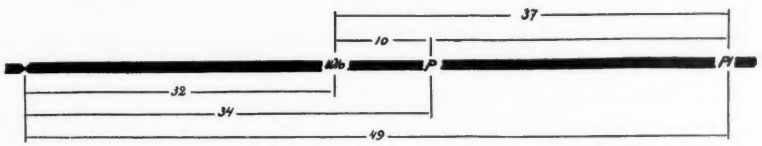

Fig. 3. The linear relation between P. i., *Wlo*, *P* and *Pl*.

That *Wlo* and *P* are connected with the N. III translocation cannot be mistaken, while *Pl* apparently shows free inheritance, which I have already demonstrated (E. NILSSON, 1933). In an earlier investigation of this linkage group, however, I showed that *Pl* (like *P*) is linked to *Wlo* (E. NILSSON, 1939), as is also evident from the following list. That *Pl* and *P* are linked was already known (WELLENSIEK, 1927; WINGE, 1936).

The following list (and Fig. 3) shows the mutual relationship of the four points (crossing-over percentage).

P. i.—*Wlo*	31,9
P. i.—*P*	34,4
P. i.—*Pl*	48,8
Wlo—*P*	10,0
P—*Pl*	30,6
Wlo—*Pl*	36,9

Hence the order of succession between *Wlo*, *P* and *Pl* previously considered as most probable by me (E. NILSSON, 1939) has proved to be correct.

GENES SHOWING FREE COMBINATION WITH THE TRANSLOCATION.

The genes of this kind under consideration here are the groups *Wb K* and *Pa Tl R* as well as *Wa*, which LAMM (1947) showed to be linked to one of the factors for plant height (Cy_2)[1] but which was not placed in any linkage group in LAMPRECHT's preliminary survey (1948). Below, the χ^2 is only given for the respective crosses in sum.

[1] *Cf.* DE HAAN (1927, 1931).

I	P. i.	*Wb*	0,2222
»	»	*K*	0,2000
IV	»	*Pa*	0,1714
»	»	*Tl*	0,6857
»	»	*R*	0,1714
V	»	»	0,0250
IX—X	»	*Wa*	0,9114

As seen, no indication of linkage can be elicited from these data. In an older paper (E. NILSSON, 1933) I showed free combination between P. i. and *Btb* (*Btb* pointless pod, *btb* pointed), which belong to the same linkage group as the above-mentioned *Pa*, *Tl* and *R*.

COMMENTS.

Summarizing the results to which these experiments have led, we find — if we adopt LAMPRECHT's grouping of the *Pisum* genes into seven groups (1948) — that a definite linkage has been established between the point of reciprocal translocation and the linkage groups, *B—St*, *Le—V—N—Fa* and *Wlo—P—Pl*, *i. e.* three groups that *loco citato* are considered to be located in different chromosomes. However, the segregation fertile: partially sterile plants gives a clear 1 : 1 ratio. As already mentioned, HÅKANSSON (1932, 1934) showed that N. III is characterized by an association of four chromosomes that is difficult to interpret and that deviates from other cases of *Pisum* semi-sterility.

In order to understand the N. III case we must find an explanation that is not in contradiction with any of these experimental facts. From the genetical point of view we then have two main paths to choose:

(1) The three linkage groups must be brought together to make two, *i. e.* they are carried in two chromosomes, though the distance between two of them is great enough to allow such ample crossing-over that the segregation gives the appearance of free combination;

(2) Three chromosomes are simultaneously translocated in this case.

According to what Dr. ROBERT LAMM, who has kindly placed his cytological experience at my disposal in the course of many discussions on this abstruse translocation case, has informed me, HÅKANSSON's (*l. c.*) N. III configuration may also appear under certain conditions in typical reciprocal translocation, and hence the visible cytological conditions do not exclude an interpretation on the lines of alternative 1.

Certain segregation figures occurring in the *Pisum* literature suggest

a possibility of co-ordinating the groups here concerned. Firstly we would point to the possibility thoroughly discussed by RASMUSSON (1927) that *P* belongs to the same linkage group as *Le—V*, a possibility that since then has been contradicted by some and corroborated by other experimental results. To the latter category of facts belong the crossing-over values found by VON ROSEN (1944) in crosses between *Pisum Abyssinicum* and lines of *P. sativum*, crossing combinations that seem to have a tendency to shorten the distances characteristic of *P. sativum*.

WELLENSIEK has published in several papers (1925, 1937, 1930) dihybrid figures for *B* and *V* that can indicate the intrachromosomal position of these genes. Arranged in the sequence *BV* : *Bv* : *bV* : *bv* these figures are as follows:

1925.....	480 : 167 : 167 : 30
1927.....	580 : 216 : 186 : 42
1930.....	587 : 185 : 219 : 42
Sum	1647 : 568 : 572 : 114

χ^2 for independence = 21,3722, P < 0,001.

Further support for these data is found in the corresponding figures for *B* and *Le* (*id.*, 1929, 1930):

1929.....	454 : 155 : 162 : 38
1930.....	431 : 174 : 149 : 39
Sum	885 : 329 : 311 : 77

χ^2 for independence = 8,1124, P = 0,01 — 0,001.

WELLENSIEK does not discuss these results in his work last cited in this connection (1930), but in a prior work (1927) he intimates — »the former results were doubtful and therefore these factors are probably independent» — that he himself does not ascribe these (nevertheless not so low) atypical dihybrid figures any importance. In a cross between the previously mentioned L. 119 (*vb*) and Extra Rapid (*VB*) I have myself, though unfortunately on too small a number of individuals, obtained the segregation (coupling phase, in contrast to WELLENSIEK):

26 : 3 : 4 : 8

χ^2 for independence = 16,0677, P < 0,001.

Still, this may very well be chromosomal linkage, as Extra Rapid is a biotype with reciprocal translocation. The same may also apply to the case with WELLENSIEK's data in spite of the fact that crosses between different materials are concerned. In some cases one of the parents is *Pisum Thebaicum*, and it may be expected that this »species» — however little of a species it is — can exhibit structural deviations. Just at present, however, nothing definite can be stated as to the connection between *Le—V* and *B*.

The following figures of LAMPRECHT (1945) may also suggest linkage between two genes that belong to two of the groups linked to P. i. in N. III, *viz. Fa* and *B* (sum of three crosses):

$$903 : 286 : 237 : 90$$

$$\chi^2 \text{ for independence} = 1{,}5198,\ P = 0{,}3 - 0{,}2.$$

The probability for dependence is not great, but the tendency of the phenotype groups to excess in the outer classes is nevertheless clear. In themselves these figures need not, statistically viewed, have been regarded as specially disturbing data if the following figures for *Fa* and *M* in the same work had not been interpretable *ex analogia*:

$$534 : 201 : 156 : 31$$

$$\chi^2 \text{ for independence} = 8{,}0216,\ P = 0{,}01 - 0{,}001.$$

B and *M* are generally supposed to belong to the same group, although crossing-over takes place with nearly 50 %. LAMPRECHT makes no comments concerning this, and I should have had no need to seek for possibilities of uniting two of the three N. III groups into one if I had not attached importance to it, and would perhaps have thereby acted more wisely. What seems to be specially worthy of attention, however, is the similar behaviour of *B—M* contra *Fa* in the coupling and repulsion phases.

Here, too, it is no doubt necessary first of all to conduct experiments in order to be able to confirm or contest the point at issue, and this also applies to the whole question as to whether the cases found of linkage with the point of interchange can be located in two chromosomes or not. The fact is that if, with the present known data at our disposal, we follow the different possibilities to their extreme consequences, we shall encounter many irritating difficulties.

The solution that the translocation concerns three chromosomes is

the easiest from a genetical point of view. The segregation 1 fertile : 1 semi-sterile can probably be established if one or the other of the translocation chromosomes has in its turn exchanged only an insignificant segment with a third chromosome. In that case it is cytologically possible to conceive of an association of four chromosomes plus a bivalent, at any rate as a common arrangement. However, I would point out that my cytological knowledge is too small for a detailed discussion of this question. Before favourizing this alternative extended investigations are under any circumstances necessary, and therefore it has happened here, as is so often otherwise the case, that a working series started with the intention of clarifying a position has become merely introductory.

As a conclusion to this paper it is appropriate to mention something about the position of the N. III translocation in relation to the previously investigated cases of reciprocal *Pisum* translocations: HAMMARLUND's case, the Thibetan translocation and Extra Rapid, *i. e.* to compare prime type E with prime types B, C and D (in order oneself to cling to an, in itself, unessential priority).

According to SANSOME (1937), B has a translocation chromosome in common with C and C one in common with D, while B and D are thought not to have any in common. From most handbooks on genetics it is known that the translocation chromosomes in B carry the genes *A* and *Gp*, and hence the original literature will not be cited here. PELLEW (1940) shows that, as was expected, *Fs* linked to *Gp* is also included in the translocation complex. LAMPRECHT (1946) shows that *Gp* is also associated with the Extra Rapid translocation (prime type D). The statement in SANSOME (*l. c.*) that B and D have no translocation chromosome in common must therefore be erroneous, which has already been pointed out by LAMM (1948, 1949). The relations should therefore be:

Prime	type	B	Chromosome	1	2	
»	»	C	»	1		3
»	»	D	»		2	3

Prime type C is according to PELLEW (1931) characterized by translocation in the *R* chromosome. In 1938 I found a distinct linkage between the P. i. in Extra Rapid and the genes *B* and *St*, both in F_2 of the cross L. 150 × Extra Rapid and in trisomic inheritance in a type that regularly occurs in Extra Rapid crosses (E. NILSSON, 1936; HÅKANSSON, 1936). The results with the trisomic type will be published

later. In the F_2 I found (the same order of succession as was mentioned earlier)

B fertility ..... 31 : 69 : 15 : 9
St » ..... 38 : 93 : 21 : 2

which figures — although they are small — I am submitting without working up, since the connection between D and the *B*-chromosome has been elucidated by LAMPRECHT (1946) and LAMM (1948).

As regards prime type E (the translocation homozygote of N. III) HÅKANSSON (1934) has shown that it has a translocation chromosome in common with D, and informed me by letter in 1940 that E × C gives a 6-ring. Hence E must also have a translocation chromosome that has the same chromosome-ends as C (the translocation homozygote of Thibetan). We can accordingly extend our earlier scheme to:

Prime type B	Chromosome	1	2		
» » C	»	1		3	
» » D	»		2	3	
» » E	»			3	4

It should, however, be observed that E may still have one more translocation.

These cytological data can be brought into agreement with the genetical by assuming that *A* and *R* are carried in the same chromosome of the normal type. By translating the cytological mode of expression to genetical symbols we then get:

Alt. I.	Prime type B	Genes	*AR*	*Gp*		
	» » C	»	*AR*		*B*	
	» » D	»		*Gp*	*B*	
	» » E	»			*B*	*Le* etc.

PELLEW has on several occasions (1931 a, 1937, 1940) also reported results with indication of *R*—*A* linkage, though without binding proof.

Another possibility is that the P. i.—*R* linkage observed in prime type C is due to the position of *R* in the B chromosome, and we then get:

Alt. II.	Prime type B	Genes	*A*	*Gp*		
	» » C	»	*A*		*BR*	
	» » D	»		*Gp*	*BR*	
	» » E	»			*BR*	*Le* etc.

In our experimental material the *R* group, however, has shown free combination with the P. i. in N. III, although this need only signify a great distance between the respective points.

Some results from a cross in 1946 between Extra Rapid (prime type D) and L. 160 (*le, pa, tl, r, btb*) deserves discussion in this connection. The F_1 of this cross and the reciprocal was back-crossed to L. 160. It was with surprise that I discovered in 1948 that *Le* on one hand and *Pa, Tl, R, Btb* on the other showed distinct linkage symptoms. The data are adduced below *in extenso*:

Loci	Numbers found	χ^2	C.-o. %
Le Pa	52 : 33 : 28 : 43	$7{,}4103^{**}$	39,1
Le Tl	51 : 34 : 31 : 40	$4{,}3333^{*}$	41,7
Le R	53 : 32 : 34 : 37	$3{,}6923$	42,3
Le Btb	48 : 37 : 31 : 40	$2{,}5646$	43,6
Le P. i.	42 : 43 : 35 : 36	$0{,}0000$	50,0
Pa P. i.	36 : 44 : 41 : 35	$1{,}2564$	45,5
Tl P. i.	37 : 45 : 40 : 34	$1{,}2564$	45,5
R P. i.	40 : 47 : 37 : 32	$2{,}9157$	46,2
Btb P. i.	37 : 42 : 40 : 37	$0{,}4103$	47,4
Pa Tl	76 : 4 : 6 : 70	$118{,}5641^{***}$	6,4
Pa R	76 : 4 : 11 : 65	$101{,}7692^{***}$	9,6
Pa Btb	48 : 32 : 31 : 45	$5{,}7693^{*}$	40,4
Tl R	82 : 0 : 5 : 69	$136{,}6410^{***}$	3,2
Tl Btb	53 : 29 : 26 : 48	$13{,}5641^{***}$	35,3
R Btb	56 : 31 : 23 : 46	$14{,}7692^{***}$	34,6

If linkage between *Le* and the *R* group is regarded as statistically proven, and of course also linkage within the previously investigated *R* group, then the crossing-over percentage *Le*—P. i. amounts to 50 and χ^2 to 0. Whether all the genes within the *R* group, which have been set up in the same order as they lie in the chromosome (not published), are linked with the point of interchange, does not admit of being statistically demonstrated, but the thorough-going and regular extravagance of the two intermediate classes is impossible to mistake. Starting from the P. i. as well as from *Le*, the genes of the *R* group arrange themselves, conformably with their distances from these loci, in accordance with their arrangement in the chromosome as elegantly as can be desired (see Fig. 4). It should thus be justifiable, at any rate as a working

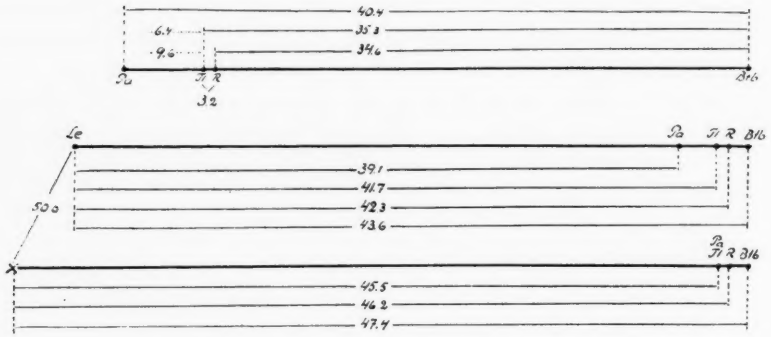

Fig. 4. Schematical view of the cross D × *le pa tl r btb*.

hypothesis, to assume that *Le* and the genes of the *R* group are linked to one or both of the points of interchange in prime type D. The data give no guidance as to whether it ought to be assumed that the two groups have each its connection with its P. i., *i. e.* whether the linkage between them found here is pseudo-linkage or whether they lie in the same chromosome in the prime type standard.

A difficulty facing the latter assumption is that no linkage between *Le* and the *R* group has been found in normal lines, but, as the translocations occasionally act as cross-over suppressors and the suppression from 50 % crossing-over to about 40 % cannot be regarded as considerable, there is scarcely any reason *on that ground* to reject true linkage between *Le* and *R*, etc. As already mentioned, N. III shows about 50 % crossing-over between the P. i. and the *R* group, but here, too, the figures exhibit an obvious tendency to $1 : > 1 : > 1 : 1$. The same applies, even if less clearly symmetrically, to *Le*—the *R* group, a relation that is also characterized by about 50 % crossing over.

Future experiments will have to clear up the gentical background to the above-demonstrated relation. However, whether it is due to a false or a true linkage, it excludes alternative I (linkage *A*—*R*) outlined above, since prime type D has chromosome 2 and not chromosome 1 (with *A*) in common with B and chromosome 3 (not chromosome 1) in common with C. There are the following arrangements to choose from (I and III do not, however, agree with the fact that a translocation point in E is linked with *Le*):

I.	B	*A*	*Gp Le*			II.	B	*A*	*Gp R*	
	C	*A*		*B R*			C	*A*		*B Le*
	D		*Gp Le*	*B R*			D		*Gp R*	*B Le*
	E			*B R*	etc.		E			*B Le* etc.
III.	B	*A*	*Gp Le R*			IV.	B	*A*	*Gp*	
	C	*A*		*B*			C	*A*		*B Le R*
	D		*Gp Le R*	*B*			D		*Gp*	*B Le R*
	E			*B*	etc.		E			*B Le R* etc.

I do not consider that I ought to discuss the possibilities that exist of interpreting the somewhat »disturbing» N. III case, as such a discussion will merely consist of making deductions with insufficient inductive support. What is first required is to increase the strength of the latter by means of further crossing analysis.

SUMMARY.

An analysis of crosses between prime type E in the reciprocal translocation N. III and normal lines with different recessive genes discloses the locus of translocation to have a distinct linkage with the mutually linked gene-groups *B—St*, *Wlo—P—Pl* and *Le—V—N—Fa* (*Le V—N Fa*; LAMPRECHT, 1948).

The arrangement is as follow:

B—P. i.—*St*
P. i.—*Wlo—P—Pl*
Le—V—P. i.—*N—Fa*.

The segregation fertile: semi-sterile does not deviate significantly from the 1 : 1 ratio, and typical semi-sterility seems to be present. In spite of this, three (possibly four) linkage groups are affected by the translocation.

The possibility of assigning these to two chromosomes or of assuming that in reality three chromosomes constitute N. III is discussed, partially with the support of data in the earlier literature.

Parallel with an account of the cytological and genetical relations between prime type E and the previously rather well-known B (HAMMARLUND's K line), C (in the Thibetan translocation) and D (Extra Rapid) types a case is reported of linkage (chromosomal or true) between the *Le* and *R* groups in the Extra Rapid translocation. The explanatory possibilities afforded by this are discussed.

The history of the literature dealing with the problem is treated.

That these studies of reciprocal translocations in *Pisum*, some results of which are submitted here, have been at all possible for me, who am not a cytologist, I have for the most part to thank Professor ARTUR HÅKANSSON, who carried out the fundamental cytological investigations of my »cases», and Dr. ROBERT LAMM, with whom I have had repeated opportunities of discussing them.

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SOME RESULTS OF A CYTOLOGICAL INVESTIGATION OF DORONICUM

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THE genus *Doronicum* belongs to the tribe Senecioneæ of the family Compositæ. A monograph of this genus has been published by CAVILLIER (1907, 1911). He described 33 species, indigenous to central and southern Europe and western Asia and confined mainly to mountainous regions. Some *Doronicum* species are cultivated as decorative plants in north Europe and occasionally occur as escapes from gardens (for example, *D. pardalianches* and *D. plantagineum* in England).

No cytological work on this genus has hitherto been published. AFZELIUS (1925) studied cytologically a large number of species belonging to eleven different genera of the tribe Senecioneæ, but no *Doronicum* species was included among these.

Material. — Difficulties were encountered in obtaining genuine material of *Doronicum* species. The first observations were made on two species, *D. pardalianches* and *D. plantagineum*, which were grown in the Botanical Garden at Lund, Sweden. It was found, however, that the first of these species had a very irregular meiosis, that it was highly sterile and that its morphology did not agree with CAVILLIER's description of *D. pardalianches*. Seed samples of various *Doronicum* species were then obtained from botanical gardens in different parts of Europe, but it was soon found that the names under which the seed was obtained could not be relied upon. Not even the name of the genus was always correct. Thus representatives of the genera *Telekia* and *Gnaphalium* were included among the samples.

The results to be dealt with here have been obtained from 3 species, the identity of which has been reliably ascertained, and also from the plants growing in the Botanical Garden at Lund under the name of *D. pardalianches*. The three species are: —

(1) *D. cordatum* SCHULTZ bip. The plants were raised from seed originating from the Botanical Garden at Berlin-Dahlem. The seed sample was obtained under the name of *D. caucasicum*, but morphologically the plants agreed completely with CAVILLIER's description of *D. cordatum*.

(2) *D. pardalianches* L. Seeds of this species were received from the Botanical Garden at the Karlowy University at Prague, although the sample was named *D. orphanidis*. The plants raised from the seed agreed in every respect with plants of *D. pardalianches* received from Kew Gardens and also with CAVILLIER's description of this species.

(3) *D. plantagineum* L. Plants of this species were also raised from seed but it is not known from which Botanical Garden the seed was obtained. The plants resembled very closely the plants of *D. plantagineum* grown in the Botanical Garden at Lund but differed from plants received from Kew Gardens under the same name. The material from Kew was taller, had darker and broader leaves and also inferior pollen fertility and seed-setting. CAVILLIER's description of *D. plantagineum* fits the plants used for this investigation but it does not fit the material from Kew.

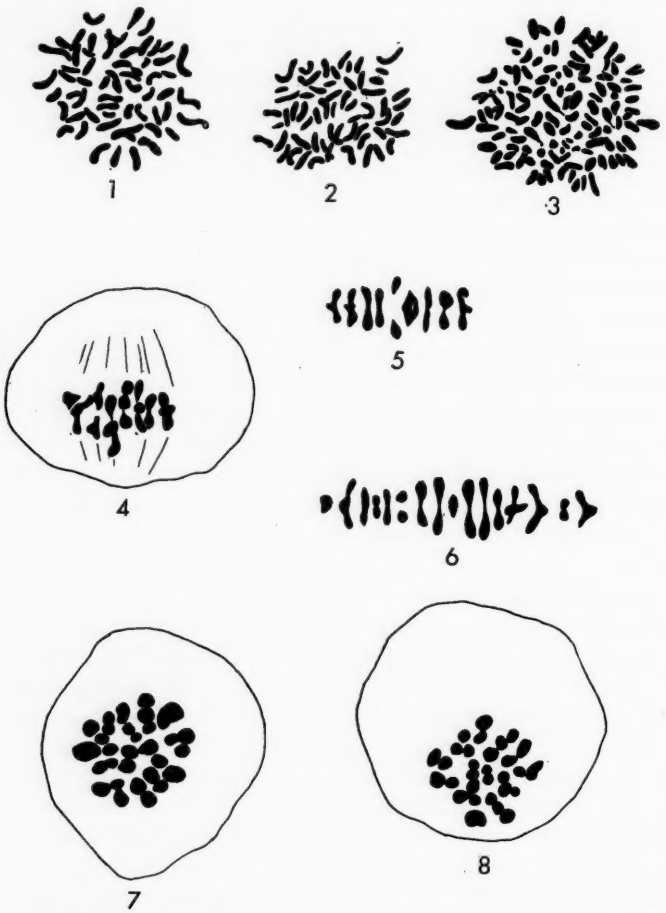
Methods. — NAVASCHIN's fixative was used for root tips. Pollen mother cells were fixed in KARPECHENKO's fixative after a few miutes pretreatment with CARNOY. All material was embedded in paraffin wax. HEIDENHAIN's hæmatoxylin was used for staining root tips as well as P.M.C.'s after it had been found that gentian violet gave unsatisfactory results.

Root tips were fixed from three seedlings of each species. These seedlings were later planted out and used also for the purpose of studying meiosis. In addition, material for the study of meiosis in *D. plantagineum* and in the plants grown under the name of *D. pardalianches*, was obtained from Lund's Botanical Garden, as mentioned above.

Results. — *Doronicum* is not a favourable object for cytological work as chromosome numbers are high and the chromosomes small. There are size differences between the different chromosomes but these are not sufficiently pronounced to make identification of the different pairs possible in somatic plates. Nor was it possible to analyse completely metaphase plates at the first meiotic division.

Mitosis. — The following chromosome numbers were found in mitotic division in root tips: *D. cordatum*, $2n = 60$; *D. pardalianches*, $2n = 60$; *D. plantagineum*, $2n = \pm 120$.

The chromosome number of the first two species can be considered as determined with certainty. The metaphase plates of *D. plantagineum* were, not surprisingly, all doubtful in one or two places, but it is fairly certain that this species has 120 chromosomes in its somatic cells. Camera lucida drawings of somatic plates of the three species are seen in Figs. 1—3. The chromosomes of *D. plantagineum* appeared to be more contracted than those of the other two species. Four of the chromosomes



Figs. 1—3. Somatic chromosomes. — 1: *D. cordatum*, $2n = 60$. — 2: *D. pardalianches*, $2n = 60$. — 3: *D. plantagineum*, $2n = \pm 120$. — Figs. 4—6. Bivalents from metaphase I in side view. — 4: *D. cordatum*. — 5: *D. pardalianches*. — 6: *D. plantagineum*. — Figs. 7—8. Metaphase I in polar view. — 7: *D. cordatum*, $n = 30$. — 8: *D. pardalianches*, $n = 30$. (Figs. 1—3, × ca. 1350; Figs. 4—8, × ca. 1750.)

in Fig. 3 have satellites. No satellites were found in *D. cordatum* or *D. pardalianches*.

Meiosis. — These three species had normal meiosis. Pairing was complete and, as far as could be ascertained, only bivalents were

formed. Figs. 4—6 show the first meiotic metaphase of the three species in side view. These plates are incomplete and are intended only to give an idea of the types of bivalents most frequently encountered. The plate in Fig. 4 had been cut by the microtome knife and in Figs. 5 and 6 only plainly discernable bivalents have been drawn. The following types of bivalents were seen: with one terminal chiasma, with one interstitial chiasma, with one interstitial and one terminal chiasma, and finally with two terminal chiasmata.

Figs. 7—9 illustrate the first meiotic metaphase of the three species in polar view. The chromosome numbers found in root tips could be confirmed here. *D. cordatum* and *D. pardalianches* were found to form 30 bivalents and *D. plantagineum* approximately 60.

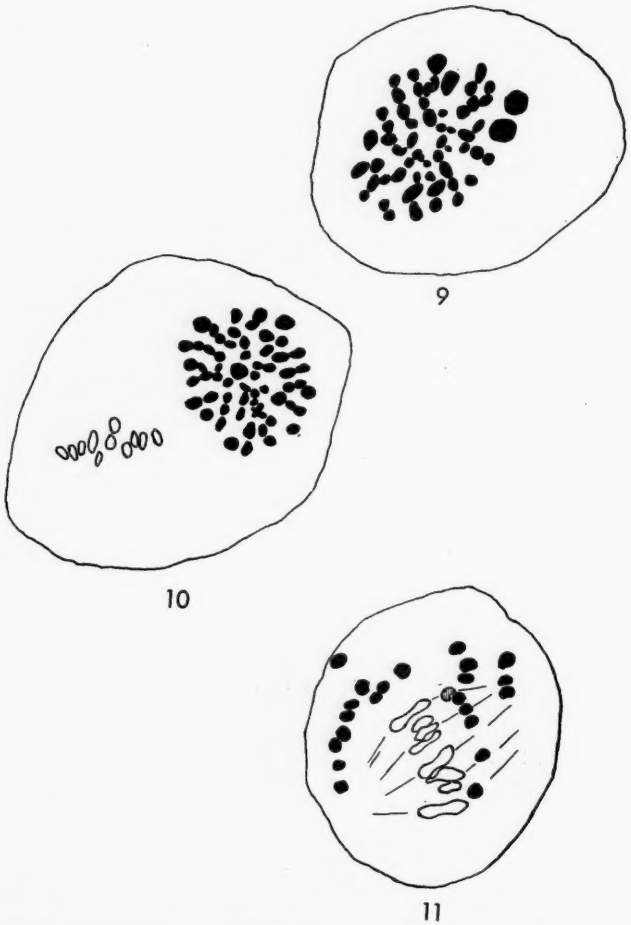
The occurrence of secondary associations was a prominent feature of these metaphase plates. In *D. cordatum* and *D. pardalianches* the bivalents had a strong tendency to lie in groups of two or three. In *D. plantagineum* groups of four, five and six bivalents were also seen. In the latter species the differences in size between different bivalents were very marked. It was therefore suspected that multivalents were formed in this species, but no certain associations of more than two chromosomes could be detected in metaphase plates in side view. Fig. 3 shows that the somatic chomosomes of *D. plantagineum* also were less uniform in size than those of *D. cordatum* and *D. pardalianches*.

No irregularities were observed in the first anaphase. The second meiotic division was also quite normal. The chromosomes were contracted almost to the same degree as at the first meiotic division. The symptoms of secondary pairing were very conspicuous also at second metaphase (Fig. 10).

All three species showed good pollen fertility and good seed setting.

The plants growing in the Botanical Garden at Lund under the name of *D. pardalianches* proved to be highly asynaptic. At metaphase I the majority of the chromosomes occurred as univalents dispersed all over the cell. Some bivalents were, however, formed in most cases and these bivalents were of the same type as those found in *D. cordatum*, *pardalianches* and *plantagineum*. The univalents had a strong tendency to lie in pairs in a way reminiscent of the secondary pairing of bivalents observed in these three species (Figs. 11—12).

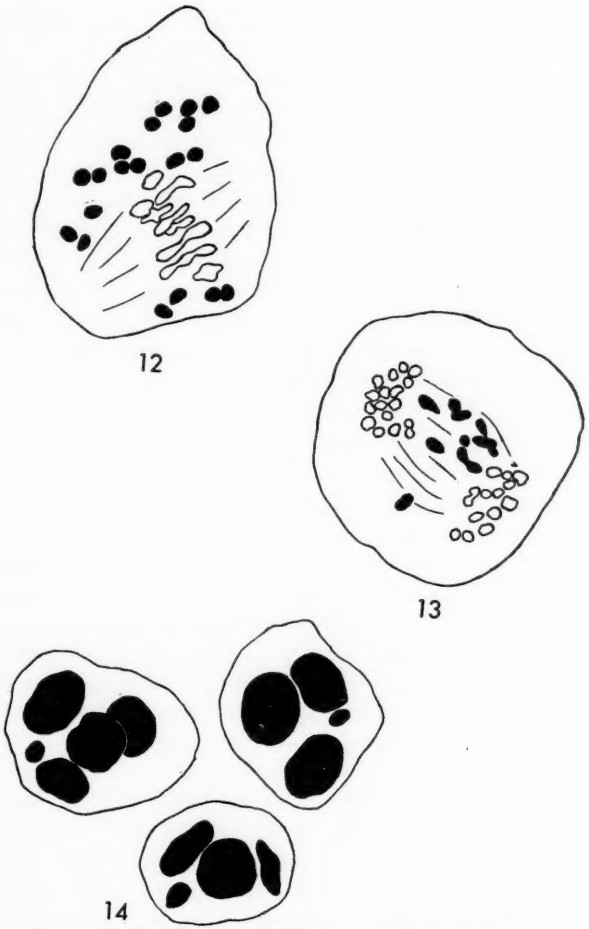
No root tips of these plants were fixed and therefore the somatic chromosome number could not be determined. It was not possible to estimate accurately the chromosome number in meiotic divisions. It can



Figs. 9—10. *D. plantagineum*. — 9: Metaphase I in polar view, $n = \pm 60$. — 10: Metaphase II. — Fig. 11. *D.* »*pardaliancheS*» from Lund. Metaphase I in side view. (× ca. 1750.)

only be stated with certainty that it was not less than 40 and not more than 60.

At anaphase I lagging univalents were observed, some apparently undergoing division. Many univalents did not become included in the daughter nuclei but formed micronuclei. Consequently the pollen tetrades often contained supernumerary small nuclei (Fig. 14).



Figs. 12—14. *D.* »*pardalianches*» from Lund. — 12: Metaphase I in side view. — 13: Anaphase I. — 14: Pollen tetrades with supernumerary nuclei. (Figs. 12—13, × ca. 1750; Fig. 14, × ca. 650.)

The slides did not contain any good fixations of the second division. As was to be expected, the plants were almost completely sterile.

Discussion. — AFZELIUS (1925) proved that polyploidy occurs within the tribe Senecioneæ. He found the following chromosome numbers $n = 5$, 10, 15, 20, 25, 30 and $\pm$ 90. A large number of species had the number $n = 30$. The same number has now been ascertained for

Doronicum cordatum and *D. pardalianches*. *D. plantagineum* with $n = \pm 60$ has one of the highest chromosome numbers so far found in the tribe. It is surpassed only by *Senecio Roberti-Friesii* with $n = \pm 90$.

Meiosis in the three *Doronicum* species studied here was characterized by the tendency of the bivalents to occur in groups of two or more. Such secondary pairing has been interpreted by DARLINGTON and his school as indicative of relationship between the chromosomes and from the occurrence of secondary pairing deductions have been made concerning the basic chromosome numbers of several genera. Thus MÜNTZING (1933) concluded from the occurrence of secondary association in diploid *Solanum tuberosum* ($n = 12$) that the original basic number of this genus is six. Similarly, LAWRENCE inferred that the basic number of the genus *Dahlia* is eight, although no species with a lower number than $n = 16$ is known at present (LAWRENCE, 1931).

As has been mentioned above, the bivalents of *Doronicum cordatum* and *D. pardalianches*, both with $2n = 60$, have a tendency to form groups of two or three, whereas associations of as many as six bivalents have been seen in *D. plantagineum*, which presumably has the somatic chromosome number 120. It is therefore possible that the basic number of this genus is ten, in which case *D. cordatum* and *D. pardalianches* are hexaploids and *D. plantagineum* dodecaploid.

The asynaptic *D.* »*pardalianches*» from the Botanical Garden at Lund was presumably a species hybrid. In a botanical garden there are excellent opportunities for natural crossing of different species. All *Doronicum* species are perennial and propagate themselves vegetatively as well as sexually. It would therefore not be surprising if species hybrids could survive and spread. The occurrence of asynapsis does, of course, not prove that the plants were hybrids. Many cases are known of asynapsis within a pure species. But the morphology of the plants also pointed to hybrid origin. The leaf shape suggested that they may have been hybrids between *D. pardalianches* and a member of the group Cardiophylla, to which *D. cordatum* belongs.

An interesting feature of meiosis in P.M.C. of these asynaptic plants was the tendency of the univalents to associate in pairs. GUSTAFSSON (1935) found in E.M.C. of *Taraxacum*, where no true bivalents are formed, that univalents formed associations which he called »pseudo-gemini». He interprets these as evidence of homology and writes as follows: »It is evident ... that the formation of pseudo-gemini is due to the fact that single homologous chromosomes happen to lie in the vicinity of each other during diakinesis and that they arise when the

repulsion between the chromosomes on the passage to metaphase decreases and the attraction becomes too powerful.»

Another case of univalents arranged in pairs has been reported by LEVAN (1945) in exceptional diploid P.M.C. in haploid sugar beet. In these diploid P.M.C. the pairing was more or less incomplete and the resulting univalents were found to lie in pairs. LEVAN does not comment on the behaviour of the univalents, but it would appear reasonable to assume that the pairs are of the same nature as GUSTAFSSON's pseudo-gemini.

Similarly, the tendency of the univalents of the asynaptic *Doronicum* plants to form pairs can be interpreted as evidence of their homology. As, however, the origin of these plants is unknown, it would be futile to speculate on the significance of this affinity between the univalents.

Although the *Doronicum* chromosomes do not lend themselves to detailed studies, this genus is worthy of a more thorough cytological investigation. A prerequisite for such work is, however, the availability of authentic material, preferably originating from the natural habitats of the species.

Summary. — The following chromosome numbers were found: *D. cordatum* SCHULTZ bip.: $n = 30$, $2n = 60$; *D. pardalianches* L.: $n = 30$, $2n = 60$; *D. plantagineum* L.: $n = \pm 60$, $2n = \pm 120$.

Secondary association at first and second meiotic metaphase suggests that the basic number of the genus is lower than 30.

I am indebted to Professor A. HÅKANSSON for advice and help and to Svenska Sockerfabriks A.B. for providing facilities for the work.

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ABSTRACTS

BENGT KIHLMAN: Induction of structural chromosome changes with adenine.

In his paper »The solubilization of polycyclic aromatic hydrocarbons by purines», WEIL-MALHERBE (1946) *int. al.* describes the solvent effect of nucleic acid purines, *viz.* adenine and guanine. He has found that they have a clear, though, compared with the methylated purines, a rather weak effect. The deamination product of adenine, hypoxanthine, was also tested, but found to be nearly inactive. In the nucleosides adenosine and guanosine, the effect was reduced to 45 per cent of that observed with the parent purine; and the corresponding nucleotides had a still weaker effect.

In a previous publication (KIHLMAN, 1949) it was pointed out that, on the whole, the same rules are valid with regard to the solvent effect of the purines as well as to their effect on chromosomes. It was thus to be expected that it should be possible to obtain chromosome changes by means of nucleic acid purines, too.

On account of their slight solubility in water, it was impossible to obtain any results with hypoxanthine and guanine. Adenine is slightly soluble, too (at 20° C the solubility of adenine is 0,09 per cent) but it is easily maintained in oversaturated solutions. It was thus possible to treat the root tips with adenine in as high concentrations as 0,54 per cent or 40 mmol/l. The experimental material was, as before, root tips of *Allium Cepa*, and the experimental and staining methods were the same as have been described in previous papers (KIHLMAN, 1949, 1950).

The experiments did not only indicate that adenine is able to induce structural chromosome changes, but also that it is possible to obtain a rather strong effect with this purine. Some representative data are brought together in the accompanying table.

Conc. mmol/l	Conc. %	Period of treatment, hours	Delay in mitosis, days	Recovery period, days	Number of anaphases counted	% A A	Fr./A
20	0,27	4	1	1 1/2	91	19,8	0,3
		12	1	2	55	21,8	0,6
40	0,54	4	1	2	95	24,2	0,7

The values indicate that, when the cells after a delay in mitosis of about one day again begin to divide, a per cent of abnormal anaphases (% AA) of about 20 may be obtained after a treatment with 40 as well as with 20 mmol/l of adenine. That the effect, however, depends on the adenine concentration and on the period of treatment, appears from the number of fragments per anaphase (Fr./A), which grows larger when the period of treatment is prolonged, or the concentration is increased. As a comparison and a measure of the strength of the effect, it can be mentioned that, with 8-ethoxycaffeine — the most efficient inducer of structural chromosome changes among the purine derivatives tested — 75 % AA and 2,8 Fr./A were obtained after a 4 hours' treatment in a 0,24 per cent solution (10 mmol/l) (KIHLMAN, 1950).

Adenine seems to act in the same way as the three 8-ethers of caffeine, ethoxycaffeine, propyloxycaffeine, and allyloxycaffeine, *i. e.*, it seems to be able to act on interphase chromosomes. The secondary effect of adenine differs, however, in some respects from the corresponding changes induced by the methylated purines tested. The chromosomes have often an eroded appearance, and attached fragments are very common. In these respects the changes probably resemble those obtained with phenols by LEVAN and TJIO (1948).

The primary effect of adenine, *viz.* that effect on the cell which can be observed immediately subsequent to the treatment, is also more complicated than that of the methylated purines. Of these changes mention will here only be made of pronounced C-mitosis as well as the occurrence of binuclear cells without chromatin bridges between the nuclei, indicating that adenine, as methylated purines, suppresses the cell-wall formation.

Some experiments with adenosine (guanosine is too slightly soluble) and with the nucleotides adenylic and guanylic acid were also made. No chromosome aberrations were, however, obtained with these compounds. With adenosine the roots were, besides other treatments, subjected to one of 8 hours in an 1,8 per cent solution (60 mmol/l).

The effect of adenylic acid has previously been tested by KODANI (1948), seeking for a component of sodium ribose nucleate capable of inducing chromosome aberrations. KODANI has namely found that solutions of sodium ribose nucleate inhibit chromosome reduplication and induce fragmentation. Adenylic acid, however, induced no such changes in KODANI's experiments, a result which is in agreement with that obtained by the present author. On the other hand, it should not be surprising if the active component should prove to be adenine.

It seems probable that the effect of adenine on chromosomes, in accordance with the effect of the methylated purines, is in some way connected with its solvent action, or with its tendency to form molecular compounds. But with regard to a nucleic acid component, other possibilities have to be taken into consideration, too.

Although it is unlikely that adenine is ever present in intracellular concentrations as high as in the experiments here mentioned, it is perhaps not quite impossible that it is responsible for spontaneous chromosome aberrations, and that some of the chemicals which induce chromosome changes may act indirectly by liberating adenine from nucleic acids.

Institute of Physiological Botany, University of Uppsala, Dec. 12, 1949.

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Y. MELANDER and E. MONTÉN: Probable parthenogenesis in *Coregonus*.

The idea proposed by MELANDER that parthenogenesis might appear in *Coregonus* caused MONTÉN to make preliminary experiments to verify this. Thus, in the autumn of 1949 the eggs from three females belonging to a morphologically distinct *Coregonus lavaretus* form named »Allgunnens djupsik» were put into hatching jars without being fertilized. In another experiment the eggs from three other females of the same kind were fertilized by spermatozoa from the different form »Allgunnens landsik» living in the same lake, Allgunnen at Aneboda, and also morphologically distinct (see THIENEMANN, 1921; MELANDER, 1946).

In the case of the unfertilized eggs it appeared after one month that about *15 % of them contain well developed embryos.* In the case of the eggs fertilized by spermatozoa from a different form nearly all of them contain embryos. The experiments have been performed by MONTÉN himself with the greatest care. The only thing that is possibly open to objection in the experimental conditions is the fact that some water from the lake where the fishes were caught was floated over the eggs immediately after they were removed from the females. If this water contained functionable spermatozoa, this might explain the development of embryos in the eggs. But the »Allgunnens djupsik» spawns at a depth of 5—6 m (MONTÉN, 1947) and it is extremely improbable that the water taken at the border of this big lake and used in the experiment should contain a concentration of functionable spermatozoa worth mentioning whether from »Allgunnens djupsik» or from »Allgunnens landsik», whose spawning period was almost entirely finished. Therefore it cannot reasonably be assumed that 15 % of the eggs were fertilized by spermatozoa from the water.

If all the fishes developing from the cross of the two above mentioned *Coregonus* forms turn out to be like their mother, the occurrence of parthenogenesis is quite certain. As soon as possible new experiments will be performed to verify these preliminary results from several points of view.

SVÄRDSON (1945) showed that polyploidy may occur in *Salmonidæ*. This increases the probability that parthenogenesis really plays some rôle in the origin of the bewildering number of forms in *Coregonus*, which has made the genus the »enfant terrible» of fish taxonomists. Perhaps *Coregonus* and certain other salmonids are partly comparable to, *e. g., Hieracium* or *Taraxacum* in plants.

Institute of Zoology, University of Lund, and Station of Fish Research, Aneboda, Ugglehult, Sweden.

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A. LIMA-DE-FARIA: Meiosis and pollen mitosis in rye under controlled conditions.

TAYLOR's work (TAYLOR, 1949 b) has the value of showing that the culture of plant tissues and organs specially developed by GAUTHERET (1942) and WHITE (1943) can be extended to anthers. TAYLOR reports the culture *in vitro* of anthers of *Tradescantia* for several hours, and shows that meiosis follows its normal course during culture.

Deviations from the normal behaviour of the chromosomes of *Tradescantia* were observed by him. He found an increase in the frequency of bivalent interlocking (TAYLOR, 1949 a) and a better means of analysing the chromosomes at pachytene (TAYLOR, 1949 b). Also in his cultures the spiral structure of the prometaphase and first metaphase chromosomes could be seen with an unusual clarity (TAYLOR, 1949 b).

While rather promising for the analysis of certain phenomena, his technique when applied to the anthers of *Tradescantia* seemed to give origin mainly to stages characterized by deviations from the normal behaviour of the chromosomes. TAYLOR considers it specially valuable as a pretreatment in studying plants with stages of meiosis difficult to analyse. This seems to be an important resource, similar to other pretreatments, but at the same time the abnormal behaviour of the chromosomes is an unfortunate side of the technique if we consider the possibility of getting the course of meiosis taking place under controlled conditions, with a normal behaviour of the chromosomes.

The present writer has decided to culture *in vitro* anthers of *Secale cereale* L. in order to study the reaction of this material to a nutrient medium.

Technique. — The technical equipment necessary to perform this work was kindly put at my disposal by Professor H. BURSTRÖM and Dr. S. ALGÉUS of the Botanical Laboratory of the University of Lund. I wish to thank Mr. A. KYLIN for his valuable help with some of the technical details associated with this investigation.

TAYLOR's technique was followed closely. The plants studied were raised from seeds belonging to the Od »land variety» of spring rye bred by the Swedish Seed Association, Svalöf. The steps to be followed in the culture of rye anthers are as follows:

(1) Determine the meiotic stage of the anthers. In rye the middle of the spike is the oldest part, and the base and the top are younger. Each flower contains three anthers that are practically at the same meiotic stage. These two facts permit an accurate knowledge of the meiotic stage of an anther occupying any position on the spike, once the stage of the circumjacent flowers has been determined. Use an iron-aceto-carmine squash for this purpose.

(2) In a sterile room excise aseptically the anthers from the flowers and place them in sterilized petri dishes containing WHITE's nutrient medium (WHITE, *l. c.*, p. 103) modified according to TAYLOR (TAYLOR, 1949 b) by adding sucrose to five parts per hundred. Before sterilization the pH of this medium is adjusted, in the case of rye, to $6{,}6 \pm 0{,}05$.

(3) Place the petri dishes in a thermostat at 25° C. No illumination was used for the study of meiosis, except in a case stated below.

(4) After the time considered necessary for the cells to reach the desired stage of meiosis has elapsed the anthers are removed from the petri dishes and permanent preparations are made with iron-aceto-carmine by the technique used for the pachytene chromosomes of rye (LIMA-DE-FARIA, 1948).

Meiosis. — Rye reacted to this nutrient medium in a different way from *Tradescantia.* The course of meiosis is regular from pachytene to tetrads, and the behaviour of the chromosomes is in most cells surprisingly normal.

After eight hours and 30 minutes of culture anthers excised at leptotene and pachytene show first metaphase to be perfectly normal. The first metaphase chromosomes exhibit one or two chiasmata per bivalent as is the normal case in rye, and the spiral structure of the chromosomes cannot be observed except in a few cells where there is a slight indication of this phenomenon. No univalents are present, nor is there indication of any pairing abnormality. First anaphase is also normal.

A perfectly normal second anaphase was observed in preparations made from another set of anthers that had been excised at pachytene and that had remained in culture for 22 hours. Normal tetrads were also present. In this culture the anthers received indirectly a small amount of light from a daylight lamp.

After six hours anthers excised at leptotene-pachytene reached pachytene. The chromosomes show a slight difference from the normal appearance. The knobs present in the pachytene chromosomes of rye (LIMA-DE-FARIA, 1948, 1949) have a bigger size in the cultured anthers and the chromomeres of the arms seem to be more distinct than in the control material. This result is similar to that obtained by TAYLOR in *Tradescantia.*

Of all the meiotic stages studied pachytene seems to be, in the case of rye, the most sensitive to the culture *in vitro.*

Pollen mitosis. — TAYLOR does not report the culture of anthers during pollen mitosis.

In *Tradescantia* the first pollen mitosis takes place inside the microspore, but the generative nucleus divides in the pollen tube during the germination of the grain.

In rye, as in other grasses, the first and second pollen mitosis take place inside the pollen grain. Both divisions proceed when the pollen grains are still enclosed in the anther.

The technique used for the culture of anthers during the pollen grain divisions followed the steps described for the study of the meiotic stages, with the exception that the thermostat was illuminated with a sodium lamp, the petri dishes receiving direct illumination from this source. The stage of the anthers was determined in the same way as for meiosis.

Owing to the fact that when placed in the nutrient medium the anthers

cannot be disinfected, a certain amount of contamination is in this way introduced into the petri dishes, which results in the development of fungi after 2—3 days of culture. In the case of meiosis, where the anthers need not be in culture for longer periods than at most 24 hours, this drawback is ignored, but in the case of pollen mitosis where the anthers remain in culture for several days, it is obviated by transferring the anthers to a new medium every other day. Cultures have been kept free from fungi by this method for 15 days.

In rye, the first pollen mitosis takes place close to the cell-wall (the axis of the spindle being parallel to the longitudinal axis of the grain) and gives rise to two nuclei of different shape and appearance and occupying a definite position in the cell: one ellipsoidal, darkly stained, that originates near the cell-wall, the generative nucleus, and another larger, spheric and faintly stained that originates in the middle of the cell, the vegetative nucleus. The vegetative nucleus remains a resting nucleus, while the generative nucleus divides once more, giving origin to two nuclei that after telophase assume spindle shape.

A set of anthers placed in culture when the pollen grains possessed a single nucleus reached first pollen mitosis after 44 hours. The chromosomes have a perfect orientation in the metaphase plate, show a contraction of the same degree as the control material, and no spiral structure is apparent. The division takes place close to the cell-wall and gives rise to two nuclei with the position, shape and staining intensity characteristic of the normal pollen grains. The differentiation between generative and vegetative nuclei is perfect. No obvious abnormalities can be seen in the cell-wall.

Four days after the beginning of the culture the same set of anthers reached the second pollen mitosis. Like the first, the second division is quite normal, and no spiral structure can be observed in the chromosomes. No definite information could be obtained concerning the degree of contraction of the chromosomes at this division. As a result of this mitosis two sperm nuclei with the characteristic spindle shape are formed and the differentiation between them and the vegetative nucleus is perfect. No pollen grains with supernumerary mitosis or other disturbances could be observed.

Discussion. — The results obtained with the culture of anthers of rye show that meiosis can be followed *in vitro* under defined conditions, the behaviour of the chromosomes being in most cells normal at first metaphase, first anaphase, second metaphase and second anaphase, and nearly normal at pachytene.

Although the technique is only in its infancy, it already shows signs of being amenable to subsequent improvements. It is not possible at present to go further than to young pollen grains, when starting from pachytene, and these grains show a cell-wall that is particularly thin. During meiosis, too, not all cells seem to continue division, and anthers of different plants have a different degree of sensitivity to the nutrient medium.

It seems desirable from now onward to speed up the process, and other details of the technique, such as the presence or absence of light, the pH and the sugar concentration of the medium, need to be investigated.

The stages of pollen mitosis are less sensitive to the culture than the meiotic stages, and the results obtained at present with them can be considered very satisfactory. However, it is unknown whether such pollen grains are able

to accomplish fecundation, which would be of particular interest if, during culture, hereditary changes could be obtained in the chromosomes by chemical treatments.

The fact that meiosis and pollen mitosis can be cultured under controlled conditions leads to the possibility of testing the action of chemicals on the mechanism and structure of the chromosomes at these stages, in a way similar to the *Allium* test developed by LEVAN and his co-workers for the study of the influence of chemicals on chromosomes and mitosis of the roots of *Allium Cepa* (LEVAN, 1949).

In various populations of *Secale cereale* there may occur a number of plants possessing B chromosomes in addition to the normal chromosome complement. Certain types of B chromosomes of rye have the ability of numerical increase by non-disjunction. This phenomenon is only known to occur at the first pollen mitosis (MÜNTZING, 1946) and at the corresponding stage in the ovules (HÅKANSSON, 1948). For this reason it was especially desirable to have pollen mitosis in rye taking place under controlled conditions, with a view to testing the response of the mechanism of non-disjunction of the B chromosomes to the action of chemical and physical agents.

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CARL A. LARSON: Phenylpyruvic oligophrenia in Swedish institutionalized mental defectives.

FØLLING's (1934 a, b) first papers on phenylpyruvic oligophrenia were followed by a paper in which FØLLING, MOHR and RUUD (1945) reported the

presence of this anomaly in about 1 per cent. in a Norwegian series of mental defectives. In another Scandinavian series (LASSEN, 1947) phenylpyruvic oligophrenia was observed in 0,98 per cent. of the Danish defectives comprised in the series.

In his American material of 20300 inmates of institutions for mental defectives JERVIS (1939) found 161 cases (0,79 per cent.) of phenylpyruvic oligophrenia. Among defectives in British institutions a percentage of 0,76 was recorded by MUNRO (1947), whilst BATES (1939) found only 3 cases of phenylpyruvic oligophrenia among 2300 English defectives.

The possibility of an uneven race distribution of phenylpyruvic oligo-

TABLE 1. *Distribution of mental defectives in institutions.*

Institution	Male defectives			Female defectives			Defectives, both sexes		
	Number examined	Cases with phenylpyruvic oligophrenia		Number examined	Cases with phenylpyruvic oligophrenia		Number examined	Cases with phenylpyruvic oligophrenia	
		Number	Percentage		Number	Percentage		Number	Percentage
Agnesfrid	17	0	0	0	0	0	0	17	0
Georgshill	34	1	2,94	38	0	0	72	1	1,39
Möllevångshem	64	0	0	84	0	0	148	0	0
Nyhem	106	2	1,90	110	0	0	216	2	0,93
Vipeholm	746	3	0,40	189	2	1,06	935	5	0,53
Total	967	6	0,62	421	2	0,48	1388	8	0,58

Agnesfrid, Georgshill, Nyhem and Vipeholm make facilities mainly for mental defectives of non-scholar grade, Möllevångshem is a training-school for educable mental defectives.

phrenia was suggested by JERVIS (1939) who examined over a thousand Jewish defectives without finding a positive case. Among his material of phenylpyruvic oligophrenia the Irish, Italian, English, German, Slavonic, Dutch, Scandinavian and mixed nationalities were represented without discernible variation in susceptibility. Neither did MUNRO (1947), who points out a certain unevenness in the geographical distribution of his British material, find any Jewish phenylketonurics. In Switzerland BRUGGER (1943) found only 1 case (0,04 per cent.) of phenylpyruvic oligophrenia in 2393 defectives.

These hints of an irregular racial or geographical distribution of phenylpyruvic oligophrenia call for further material for comparison. A Swedish series of 1388 institutionalized mental defectives, examined with the ferric chloride test, is therefore presented here.

The series was collected from 5 institutions for mental defectives of which the largest, Vipeholm State Hospital, receives patients from all parts of Sweden whereas the other institutions admit patients from the south of the country only. The results are recorded in Table 1 and compared with those obtained by other workers in Table 2.

The clinical picture will not be dwelt on here, a few characteristics of

the positive cases will however be mentioned in connection with the probably unique report on the brain histology of one of the cases and the more trivial histology of another.

Case 1: A 20-year-old male idiot (Georgshill). Hair: fair, 22 on FISCHER scale. Eyes: blue, 15 on MARTI scale.

Case 2: A 31-year-old male idiot (Nyhem, file number 484). Hair: fair, 26 on FISCHER scale. Eyes: blue, 8 on MARTI scale.

Case 3: A 33-year-old male low grade imbecile (Nyhem, file number 485).

TABLE 2. *Comparison of the frequency of phenylpyruvic oligophrenia in various series.*

Author	Material	Number examined	Positive cases Number	Positive cases Percentage
BATES, 1939	English institutionalized mental defectives	2300	3	0,13
BRUGGER, 1943	Swiss mental defectives from institutions and auxiliary schools	2393	1	0,04
FØLLING, MOHR and RUUD, 1945	Norwegian mental defectives, 1/3 of them institutionalized	c. 2400	29[1]	c. 1,21
JERVIS, 1939	American institutionalized mental defectives	20300	161	0,79
LARSON, 1950	Swedish institutionalized mental defectives	1388	8	0,58
LASSEN, 1947	Danish institutionalized mental defectives	830	8	0,98
MEDLICOTT, 1944	Institutionalized mental defectives in New Zealand	190	2	1,05
MUNRO, 1947	British institutionalized mental defectives	4090	31	0,76

[1] To enable comparison 5 positive cases found among relatives of known cases of phenylpyruvic oligophrenia have been excluded here.

Brother of the patient in case 2. Hair: fair, 24 on FISCHER scale. Eyes: blue, 15 on MARTI scale.

Case 4: A 20-year-old female idiot (Vipeholm, file number 48/45). Hair: fair. Eyes: blue. Died from perforating ventricular ulcer. Weight of brain: 1100 g. The brain was examined by Professor NILS GELLERSTEDT, M. D., Brain Laboratory, Institute of Pathology, Upsala, with the following result: »A notable cytoarchitectonic disturbance is seen most manifestly in area 6 of the frontal lobe, consisting of an uncommonly dense and uniform cytoarchitecture in the deepest cortical layers, *i. e.* 5—6, which appear to be fusing together.

These changes are seen in the top regions of the gyri, no cortical disturbances are manifest in the depths of the sulci or in the side areas of the gyri. The significance of these findings is uncertain. They may be due to developmental arrest. In addition hereto a few malformed or misplaced nerve cells are seen in the cortex, the latter findings are often seen in brains from idiots.»

Case 5: A 21-year-old female idiot (Vipeholm, file number 10/41). Hair: fair. Eyes: blue. Died from pulmonary and abdominal tuberculosis. Weight of brain: 1000 g. Histological examination (Professor GELLERSTEDT): Sparse and banal changes of type common in brains from idiots.

Case 6: A 37-year-old male idiot (Vipeholm, file number 214/36). Hair: fair, 24 on FISCHER scale. Eyes: blue, 15 on MARTI scale.

Case 7: A 19-year-old male idiot (Vipeholm, file number 47/46). Hair: fair, 22 on FISCHER scale. Eyes: brownish gray, 6 on MARTI scale.

Case 8: A 9-year-old male idiot (Vipeholm, file number 75/49). Hair: fair, 22 on FISCHER scale. Eyes: blue, 12 on MARTI scale.

Of these 8 cases of phenylpyruvic oligophrenia numbers 1, 2, 3, 5, and 8 were born in Scania, Bleking and Smolandia in the south of Sweden, numbers 4 and 7 in the county of Gothenburg and Bohus in south-western Sweden and number 6 in Stockholm of parents from northern Sweden.

The frequency of phenylpyruvic oligophrenia in this series is in accord with the findings of American, British and Scandinavian investigators.

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